# Phylogenetics and biogeography of the relhanioid paper-daisy lineage (Asteraceae: Gnaphalieae): palaeoenvironmental evolution, migration and speciation in southern Africa and the greater Afrotemperate regions

Joanne Bentley

Thesis presented for the degree of Master of Science in the Biological Sciences Department, University of Cape Town.

January 2014

Supervisors:

Assoc. Prof. G. A. Verboom

Department of Biological Sciences, UCT

Dr N. G. Bergh

South African National Biodiversity Institute

## Declaration

I, Joanne Bentley, hereby: (a) grant the University of Cape Town free license to reproduce the above thesis in whole or in part, for the purpose of research; (b) declare that: (i) the above thesis is my own unaided work, both in conception and execution, and that apart from the normal guidance of my supervisor, I have received no assistance apart from that stated below; (ii) neither the substance nor any part of the above thesis has been submitted in the past, or is being, or is to be submitted for a degree at this University or at any other University, except as stated below; I am now presenting the thesis for examination for the Degree of Master of Science.

I declare that Chapter Four of this thesis has already been accepted for publication in a peer-reviewed scientific journal (BMC Evolutionary Biology). The work in the published chapter was performed under close supervision, and I am the principal author. The chapter has, however, been modified substantially from that accepted for publication, by myself, to improve cohesion and flow with the other chapters of this thesis.

Joanne Bentley, January 2014.

## Acknowledgements

I would like to thank my supervisors, Assoc prof Tony Verboom and Dr Nicola Bergh, for their tireless support and comment on this dissertation. Both have provided invaluable knowledge and expertise in their own right, and have been willing and able to assist and guide consistently, on top of the demands of their busy schedules. At the same time, I thank them for encouraging me to think independently and allowing the dissertation to follow in my vision, that vision however, not being possible without their rational guidance.

This research was made possible by the Knowledge Fields Development (South African Biosystematics Initiative) grant of the National Research Foundation awarded to Dr Nicola Bergh, as well as the postgraduate entry level bursary, NRF extension and Dorothy Cameron Scholarships awarded to myself.

Various other people and institutions made important contributions to this body of work. I therefore thank Awot Gebregziabher for her many hours of georefencing and Tony Abbott who gave valuable information regarding the location of a particularly elusive plant and the PRU, PRE, NU, BOL, NBG, K, MO and Madagascar Biodiversity Centre herbaria for providing specimens and leaf material. Thanks to Jack Viljoen for his extensive assistance with R and Esmeralda Klaassen for contributing molecular sequences. I also thank my field assistants Christine Cozien, Carly Appleby, Craig McKune, Tyrel Flügel, Terri McFarlane and Micheala Stehr for the many strained hours they contributed to finding plants, in all weather conditions. I also thank Vera Hoffmann, Matthew Britton, Terry Trinder-Smith and Tim Moore for their help at Ingeli Mountain. Thanks to Sandy Smuts and Tamara Nozewu for answering my endless administrative questions. Finally, I thank Craig McKune for tolerating my thesis-related anxiety and stress, and for inspiring me endlessly. Thanks to Terri McFarlane for her enthusiasm and encouragement. Thanks to my mother, Colette Bentley, for making things possible (“thoughts become things”).

**Table of Contents** **Page**

**List of Tables**



**List of Figures**

**List of Appendices**

# Chapter One: General introduction

Gnaphalieae is a moderately-sized tribe in the largest family of flowering plants, the Compositae, containing about 1240 species in roughly 190 genera (Bayer et al. 2007; Ward et al. 2009). Gnaphalieae has a more-or-less cosmopolitan distribution but most of the diversity is housed in Australia and New Zealand, and in southern Africa. Gnaphalieae are known as "paper daisies" or "everlastings", due to their defining feature of papery, sterile, often colourful and attractive involucral bracts surrounding the capitulate inflorescence. The true circumscription and subtribal division of Gnaphalieae is currently not known, and a series of recent molecular phylogenetic analyses have indicated that the previously-accepted subtribal delimitation of Anderberg (1991) comprises non-monophyletic entities (Bayer et al. 2000; Bergh & Linder 2009; Ward et al. 2009). Anderberg's (1991) morphological parsimony analysis identified five subtribes, including the largely southern African subtribe Relhaniinae, considered by Linder (2003) to be the tenth-largest "Cape Floral Clade", as well as a non-classified group of taxa which he considered putatively "basal" to Gnaphalieae. A subsequent molecular analysis (Bayer et al. 2000) indicated that many of the taxa from Anderberg's (1991) subtribe Relhaniinae and the southern African members of the "basal taxa" constitute a single lineage. This lineage, termed the "*Relhania* clade" by Bergh & Linder (2009), is identified by several studies as the earliest-diverging lineage in Gnaphalieae (Bayer et al. 2000; Bergh & Linder 2009; Ward et al. 2009). The *Relhania* clade (whose members will hereafter also be referred to as "relhanioid" taxa) forms the focus of this thesis. To date, only placeholder representatives of relhanioid species have been represented in phylogenetic analyses (Bayer et al. 2000; Bergh & Linder 2009; Klaassen & Bergh 2012; Montes-Moreno et al. 2010; Smissen et al. 2011; Ward et al. 2009); thus the *Relhania* clade as currently defined comprises only 24 species. On morphological grounds, the following taxa are likely to be relhanioid: *Antithrixia* (monotypic), *Arrowsmithia* (monotypic), *Athrixia* (14 sp.), *Comborhiza* (ditypic), *Leysera* (3 sp.), *Macowania* (12 sp.), *Nestlera* (monotypic), *Oedera* (18 sp.), *Oreoleysera* (monotypic), *Pentatrichia*

(6 sp.), *Phagnalon* (41 sp.; three of which are subspecific), *Relhania* (13 sp.), *Rhynchopsidium* (2 sp.) and *Rosenia* (4 sp.). If all these taxa are confirmed to be members of the *Relhania* clade, the group will comprise a total of 119 species in 14 genera. Two further genera, *Alatoseta* (monotypic) and *Philyrophyllum* (ditypic) are believed to be closely-related to, if not members of, the *Relhania* clade (Anderberg 1991; Montes-Moreno et al. 2010) although they are morphologically anomalous taxa whose relationships are poorly understood.

Relhanioid species are perennial shrubs, sub-shrubs or annual herbs exhibiting high morphological variation. The inflorescence is capitulate and consists of florets borne on a contracted receptacle, this being surrounded by a protective involucre of sterile, mostly dry bracts. The presence of ray florets in most members differentiates them from the rest of Gnaphalieae, though some have discoid or the common gnaphalioid disciform capitula. When present, the ray florets vary in colour from white, yellow or orange to pink-purple, or may also have different colours on the abaxial and adaxial surfaces. The capitulum may be solitary, paired or congested, and a few species possess an unusual secondarily-compound head surrounded by a pseudo-involucre of leaves. The involucre in most other species is dry and papery or rarely cartilaginous, with brownish, transparent or brown-and-white lamina. Leaf-shape varies from linear to orbicular with margins that are either smooth, minutely-toothed or strongly dentate. Toothed or incised margins also separate some members of the group from the rest of Gnaphalieae which is universally characterised by entire leaf margins. The leaves in the *Relhania* clade are mostly concave and involute but may be revolute, and are generally tomentose, usually adaxially so, and often glandular-hairy with multicellular glands. Many species have a pappus of barbellate or plumose bristles and/or an outer row of scales; in some taxa the pappus is completely lacking.

The group has a broad pan-African geographic range spanning predominantly temperate and low-humidity environments. Habitats range from highly mesic (> 2,000 mm mean annual precipitation:

MAP) to extremely arid (< 100 mm MAP) with varying rainfall seasonality. There are three main centres of relhanioid species diversity: the Greater Cape Floristic Region (GCFR: Born et al. 2007; Jürgens 1997), and the Afrotemperate (defined below) Drakensberg mountain range, both in southern Africa, and the greater Mediterranean region. In southern Africa (Fig. 1), most species (38% of the total) inhabit the predominantly winter-rainfall GCFR. The Drakensberg mountains, situated along the eastern Escarpment (defined below) of South Africa, hosts 19% of relhanioid species, comprising the third largest centre of diversity. The second largest centre of diversity (34% of species, mostly in the genus *Phagnalon*) extends from the Mediterranean Basin across arid northern Africa including Macronesia, the Irano-Turanian region and the Saharo-Arabian region; for the purpose of this dissertation, the sum of these regions will be referred to as the "circum-Mediterranean" region. Two additional smaller centres of diversity exist, one throughout the rest of the Afrotemperate archipelago (extending from the Chimanimani mountains of Zimbabwe, through to the highlands of Madagascar, Malawi, Kenya, Ethiopia, Eritrea and Yemen), hosting 7% of relhanioid species. The other occurs in the summer-rainfall, semi-arid regions of the Nama Karoo Biome extending northwards into the Namib Desert in Namibia (7% of species).

One of the primary aims of this thesis is to produce a robust, fully-sampled phylogenetic hypothesis for the *Relhania* clade, in order to ascertain its membership, the circumscription of member genera, and the relationships within and amongst genera. This phylogeny will then be used to fulfill another main aim, which is to explore the biogeographic origin and evolution of relhanioid lineages in the context of the known tectonic and climatic history of Africa and particularly, southern Africa. While the radiation of flora in the GCFR and circum-Mediterranean is comparatively well-studied using phylogenetic methods, the Afrotemperate Drakensberg region is less known. In particular, the use of phylogenies to study biogeography and speciation in the Drakensberg is in its infancy. A substantial part of this study is thus aimed at understanding the processes which might have set the Drakensberg as an important element in the radiation of relhanioid lineages. A review of the

palaeoenvironmental history of these regions, including an assessment of possible historical routes of connectivity, is necessary to understand the present-day diversity of these lineages, and is presented below.

The pan-African distribution of the *Relhania* clade is marked by large geographic range disjunctions in both arid and Afrotemperate-adapted lineages (Fig. 1). This is not surprising as a remarkable degree of shared genera exist in many plant lineages between the arid regions of southwestern Africa and the circum-Mediterranean region (e.g. Bellstedt et al. 2012; Carlson et al. 2012; Coleman et al. 2003; Jürgens 1997; Thiv et al. 2011). The flora of the Afrotemperate regions (Linder 1990), a combination of White's Cape phytochoria (1983) and Afromontane regions (1978), occurs in the Cape (where it is confined to isolated patches in fire-protected ravines and cliffs), the Drakensberg as well as the East African montane archipelago. Across this distribution, it exhibits homogeneity in ecology and phytogeography (Linder 1990). Hilliard & Burtt (1987) estimated that 22% of the genera occurring in the southern Drakensberg have Cape-centred distributions. Also, many Drakensberg and Cape genera have members distributed northwards across Afrotemperate East Africa (Hilliard 1978; Linder 2003; Taylor 1978; Uys & Kron 2013). Much of the flora of the Afrotemperate East African regions is thus believed to be derived from the Cape and the Drakensberg (Galley et al. 2007; Hilliard & Burtt 1987; Linder 1990). The high degree of homogeneity within each of the Afrotemperate and arid regions has led to the suggestion that closely-related lineages may have migrated along specific routes within the confines of their environmental niche. For arid lineages, historical arid “tracks” (Fig. 1: Balinsky 1962; Jürgens 1997), or corridors of continuous or semi-contiguous habitat, are believed to have existed periodically since the Miocene (e.g. Bellstedt et al. 2012; Caujapé-Castells et al. 2001; Coleman et

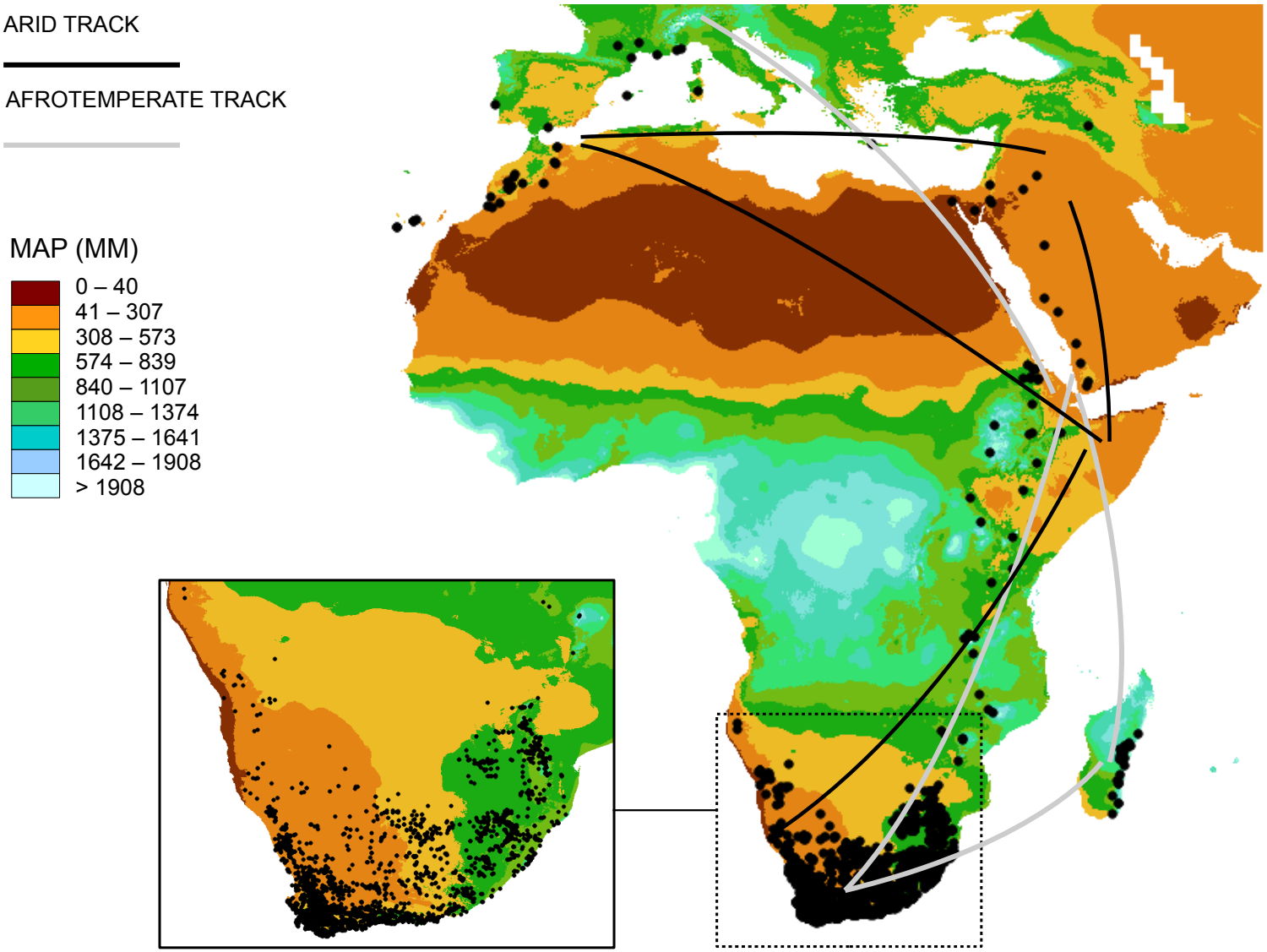



**Fig. 1. Map indicating the pan-African distribution of *Relhania* clade species (black dots) and zones of different mean annual precipitation (MAP) obtained from BIOCLIM (variable 12).**
The black and grey lines indicate hypothesised biogeographic corridors (the arid and Afrotemperate tracks). The black dots represent data obtained from georeferenced specimens from South African herbaria (BOL, NBG, NU, PRE, PRU) and from online herbarium sheet records (JSTOR Global Plants, Kew & Paris online digital herbaria) for taxa with distributions outside of southern Africa.

al. 2003; del Hoyo et al. 2009; Thiv et al. 2011), facilitating migration between distant regions of similar habitat. Afrotemperate lineages are hypothesised to have dispersed along high mountain peaks or "sky-islands", specifically the Drakensberg, acting as migratory "stepping-stones" (Galley et al. 2007), though few studies have explored this. Species might be expected to track their ecological niches along these tracks (Kozak & Wiens 2006; Wiens 2004; Wiens et al. 2010), and as has been demonstrated previously (Schrire et al. 2005; Thiv et al. 2011), widely-distributed clades should occupy analogous habitats in distant areas.

Studies of Gnaphalieae (including the *Relhania* clade) and other lineages from the daisy subfamily Asteroideae have found that members typically originate in southern Africa, from where they disperse, seeding radiations in the Mediterranean, Australia and Americas. This indicates that for the daisy family, southern Africa has played an important role as an "evolutionary springboard" in the northwards radiation and diversification of these lineages (Bergh & Linder 2009; Funk et al. 2005; Himmelreich et al. 2008; Oberprieler 2005; Pelser et al. 2007). Studies have identified the GCFR in particular as the likely centre of origin for some pan-African lineages (e.g. del Hoyo et al. 2009; Galley et al. 2007; Linder 2003). The GCFR hosts exceptional specific endemism at 69% (Cowling et al. 1992; Goldblatt 1978; Goldblatt & Manning 2002), and this is partly explained by its island-like isolation as it is bounded by oceans, mountains or hyper-arid regions (Linder 2003). The Middle Miocene climate of the GCFR is thought to have been mostly wooded and sub-tropical (Coetzee 1978, 1983; DuPont et al. 2011), so ancestral lineages are believed to have been located in the Cape Fold mountains (Linder et al. 1992; Verboom et al. 2009). These ancient mountains, in existence since the Early and Middle Triassic (Partridge & Maud 1987), are comprised of nutrient-poor quartzites and are likely to have offered a temperate refugium from the subtropical lowland climate (Linder et al. 1992). The development of summer-arid conditions from the Late Miocene (since at least 11 Ma), due to the establishment of the Benguela current (DuPont et al. 2011; Marlow et al. 2000; Partridge 1998; Siesser 1978; Tyson & Partridge 2000) along with the drying-

effects of global cooling of ocean temperatures, ultimately blocking summer-rainfall in the region (Shackleton & Kennet 1975), is believed to have prompted the contraction and large-scale extinction of the sub-tropical flora. Possibly, this allowed the radiation of refugial lineages into the newly-available lower-lying areas (Linder et al. 1992; Linder 2003; Verboom et al. 2009).

Like much of the GCFR, the floristically-diverse (Cowling et al. 1996; Quézel 1978) circum-Mediterranean region is characterised by mild, wet winters and hot, dry summers, and the aridification of the circum-Mediterranean region, estimated to have commenced since the Late Miocene (9 – 8 Ma), has also led to the extinction of historical sub-tropical elements and their replacement by sclerophyllous plant communities (Agusti et al. 2003; Axelrod 1975; Ivanov et al. 2002; Thompson 2005).

The GCFR is bordered inland by the arid Nama Karoo, a region land-locked upon a central feature of the southern African landscape: the raised interior plateau of the Great Escarpment (Partridge & Maud 1987). Initiated during the Late Jurassic and Early Cretaceous through rift-faulting (where the Earth's crust and lithosphere are pulled apart), which broke up Gondwanaland (Partridge & Maud 1987), the Great Escarpment is thought to have experienced numerous cycles of tectonic uplift (geologic uplift of the Earth's surface) followed by landscape erosion and rejuvenation (Burke & Gunnell 2008; King & King 1959; Partridge 1998; Partridge & Brink 1967; Partridge & Maud 1987, 2000), which have sculpted its heterogeneity. Asymmetric tectonic uplift during the Miocene (± 18 Ma) and Pliocene (± 5 Ma) is suggested to have further raised the Great Escarpment approximately 1150 m along the eastern margin and about 250 m in the west (Partridge & Maud 1987). The upland eastern margin of South Africa contains a significant geologic feature, namely, the Drakensberg mountain range, with much of its eastern extent elevated above 2000 m and its highest peak at 3482 m. The Drakensberg encompasses a substantial part of the high-altitude eastern Escarpment of South Africa, and the greater elevation of the range, relative to the rest of the

Great Escarpment, has been attributed to pronounced upward flexing in response to local intense erosion on one flank (Burke & Gunnell 2006; Gilchrist & Summerfield 1994; Lageat & Robb 1984). Various lines of evidence suggest that the speciation events leading to the endemics of the Drakensberg are relatively young, probably having commenced within the last five Ma (Devos et al. 2010; Galley et al. 2007; Verboom et al. 2003). The region is relatively diverse and the Drakensberg Alpine Centre (DAC), a high-altitude centre of endemism, hosts 16% endemism of flowering plant species (Carbutt & Edwards 2004). Another regional centre of endemism exists in the archipelago-like Afrotemperate highlands of East Africa, whose flora is distinct in structure and composition from that of the surrounding lowlands (White 1978). These sky-islands, situated along the East African rift system (a series of several thousand kilometre-long rift valleys separated from each other by shoals and uplifted shoulders), are the product of rift-flank uplift (flexing of the Earth's surface associated with tectonic rifting). Rifting is thought to have occurred during several intervals from the Middle to Late Miocene in the region of Ethiopia (from ± 11 Ma) and somewhat later, in the Late Miocene and Pliocene/Pleistocene (from ± 8 – 2 Ma), in the southern extent of the region (Chorowics 2005; Flannery & Rosendahl 1990; Wolfenden et al. 2004).

## Summary of aims

### Chapter Two: Phylogenetic hypothesis for the *Relhania* clade

Molecular phylogenies of the recent past (e.g. Bayer et al. 2000; Bergh & Linder 2009; Montes-Moreno et al. 2010; Smissen et al. 2011; Ward et al. 2009) have signalled the existence of the *Relhania* clade through the use of a few placeholder species for each of the genera, though the generic and species-level relationships in the clade have not been rigorously assessed. Thus, a comprehensive, species-level molecular phylogeny is currently lacking for the *Relhania* clade. Addressing this gap, the aim of this chapter is to assemble a rigorous phylogenetic hypothesis for the *Relhania* clade, including a range of outgroup taxa and all putative member taxa, and use this to

evaluate and, where necessary, rearrange generic boundaries. The phylogenetic hypothesis for the *Relhania* clade will be based on multiple nuclear and plastid DNA markers, with node support examined via parsimony bootstrap and Bayesian methods of phylogeny reconstruction. While DNA sequence data provides huge phylogenetic power, adaptations primarily impact the phenotype, thus morphology will be used as an additional source of phylogenetic data to complement the molecular analysis, these being identified using published literature.

**Chapter Three: Estimating pathways of dispersal: the palaeoenvironmental migration of Afrotemperate and arid relhanioid lineages**

The *Relhania* clade is well-represented in the Afrotemperate and arid regions of Africa and the circum-Mediterranean. Several range disjunctions along putative arid and Afrotemperate tracks are also evident, presenting opportunities to test the migration scenarios underlying these disjunctions. Modern Bayesian methods of phylogenetic inference provide a powerful means of estimating species relationships, and dating the successive speciation events underlying present-day species diversity by means of a molecular clock. Likelihood-based biogeographic analysis as implemented in Lagrange (Ree et al. 2005; Ree & Smith 2008) provides a framework for testing alternative hypotheses relating to the origin and directionality of dispersal, using a dispersal-extinction-cladogenesis (DEC) model of lineage evolution. Using these tools, several hypotheses relating to the biogeography of the *Relhania* clade will be tested, including: a Cape origin and northwards directionality of dispersal; that Afrotemperate and arid lineages disperse via separate, distinct "stepping-stones" or continuous/semi-contiguous corridors; and that dispersal along these tracks commenced with little change in the moisture niches of the lineages involved.

**Chapter Four: Erosive processes after tectonic uplift stimulate vicariant and adaptive speciation: evolution in the Drakensberg near-endemic genus *Macowania***

The role of tectonic uplift in stimulating speciation in South Africa's only alpine zone, the

Drakensberg, has not been explicitly examined. Tectonic processes may influence speciation both through the creation of novel habitats and by physically isolating plant populations. The Afrotemperate endemic relhanioid genus *Macowania* is used to explore the timing and mode of speciation (geographic versus adaptive) in this region. Between sister species pairs, high morphological divergence is expected where speciation has happened in sympatry (adaptive) while with geographic (vicariant) speciation one may expect to find less morphological divergence and a greater degree of allopatry. A dated molecular phylogenetic hypothesis for *Macowania* (obtained in Chapters Two and Three) elucidates species' relationships and is used to address the potential impact of uplift on diversification. Morphological divergence of a small sample of reproductive and vegetative characters, used as a proxy for adaptive divergence, is measured against species' range distributions to estimate mode of speciation across two subclades in the genus; one sublclade associating largely with dry, scarp-edge habitats, and the other inhabiting a greater range of ecological situations.

# Chapter Two: Phylogenetic hypothesis for the *Relhania* clade

## Introduction

Phylogenetic classification is based primarily on the principle of taxon monophyly *sensu* Hennig (1950, 1965); in other words, a taxon corresponds to a clade consisting of an ancestor and all of its descendants. Over the years, taxonomists have increasingly used monophyly as the key criterion for the delimitation of taxa, and when paraphyletic taxa are encountered, these are re-delimited in a way that renders them monophyletic (Backlund & Bremer 1998). The benefits of a monophyly-based classification system are: firstly, that it is based on shared ancestry and thus tends to be biologically predictive (i.e. closely-related taxa might share properties such as morphology and behaviour); secondly, that it reflects evolutionary reality; and thirdly, that it allows for the identification of attributes which have arisen independently rather than as a result of inheritance from a common ancestor (convergence).

Prior to the development of novel technologies for characterising DNA sequences, morphology was the primary source of information employed in classification. Although single or few diagnostic key morphological characters were initially used to classify taxa, the development of phenetic and phylogenetic analytical methods through the advent of intensive computational equipment, facilitated consideration of greater numbers of morphological characters associated with larger, multiple-character datasets. The further development of polymerase chain reaction (PCR) allowed for information available in larger, multiple-character nucleotide datasets to be harnessed for phylogenetic inference, minimising the idiosyncrasies associated with smaller datasets. It has been suggested that DNA is better in informing phylogenies than morphology owing to its greater consistency, as the choice and coding of morphological characters are subject to the authors' interpretation, and the number of characters for a given dataset are in most cases fewer (Givnish & Sytsma 1997). Levels of homoplasy (the sharing of characters for reasons other than common

descent, such as convergence, parallelism or reversal) also tend to be higher in analyses that use morphological characters compared with those using DNA (Givnish & Sytsma 1997; Hedges & Sibley 1994; Scotland et al. 2009; Wiens 2001), though whether this is due to convergence or character misclassification is not clear. In many cases, DNA and morphological data may prove to be mutually complementary (e.g. Doyle et al. 1994; Eldenäs & Linder 2000; Sanderson & Donoghue 1989). Though the use of DNA data will unavoidably produce a more precise phylogeny in most cases (Givnish & Sytsma 1997), morphology remains important in diagnosing taxa.

The purpose of this chapter is to evaluate generic limits within the *Relhania* clade using monophyly as a primary grouping principle. The first major modern analysis to set the scene for the current delimitation of the *Relhania* clade was a parsimony-based analysis of 82 morphological characters for the entire tribe Gnaphalieae using genera as terminals (Anderberg 1991). Anderberg identified five subtribes and a group of unassigned, putatively 'basal' genera. Within subtribe Relhaniinae he recovered a "*Relhania* group", consisting of *Antithrixia, Oreoleysera, Leysera, Rosenia, Relhania* and *Oedera.* His unassigned genera included *Alatoseta, Aliella, Anisothrix, Athrixia, Macowania, Pentatrichia, Phagnalon, Philyrophyllum* and several others (see Fig. 2.1). The first molecular study of South African Gnaphalieae was a parsimony analysis based on the *trnL-F* marker (Bayer et al. 2000). While the tree generated by this study has few well-supported nodes, it shows that most of Anderberg's (1991) "basal" elements together with his "*Relhania*" group constitute a single lineage which is sister to the rest of Gnaphalieae (Fig. 2.1). This lineage corresponds to what is now commonly termed the "*Relhania* clade" (firstly by Bergh & Linder 2009). Several years later, the first multi-locus, model-based analysis of Gnaphalieae was presented by Bergh & Linder (2009), who used both nuclear and plastid markers and included both African and Australian representatives of Gnaphalieae. The results of their analyses were largely consistent with that of Bayer et al (2000) and were further substantiated in a plastid DNA genus-level study in which the *Relhania* clade was recovered as monophyletic and sister to the rest of Gnaphalieae (Ward et al.

2009). A recent *trnL-F* analysis (Montes-Moreno et al. 2010) of the Northern Hemisphere genus *Phagnalon* (Montes-Moreno et al. 2013) showed that its forms a clade with *Pentatrichia;* this clade is sister to *Athrixia.* This larger clade is sister to a clade containing the remaining representatives of the *Relhania* clade (Fig. 2.1).

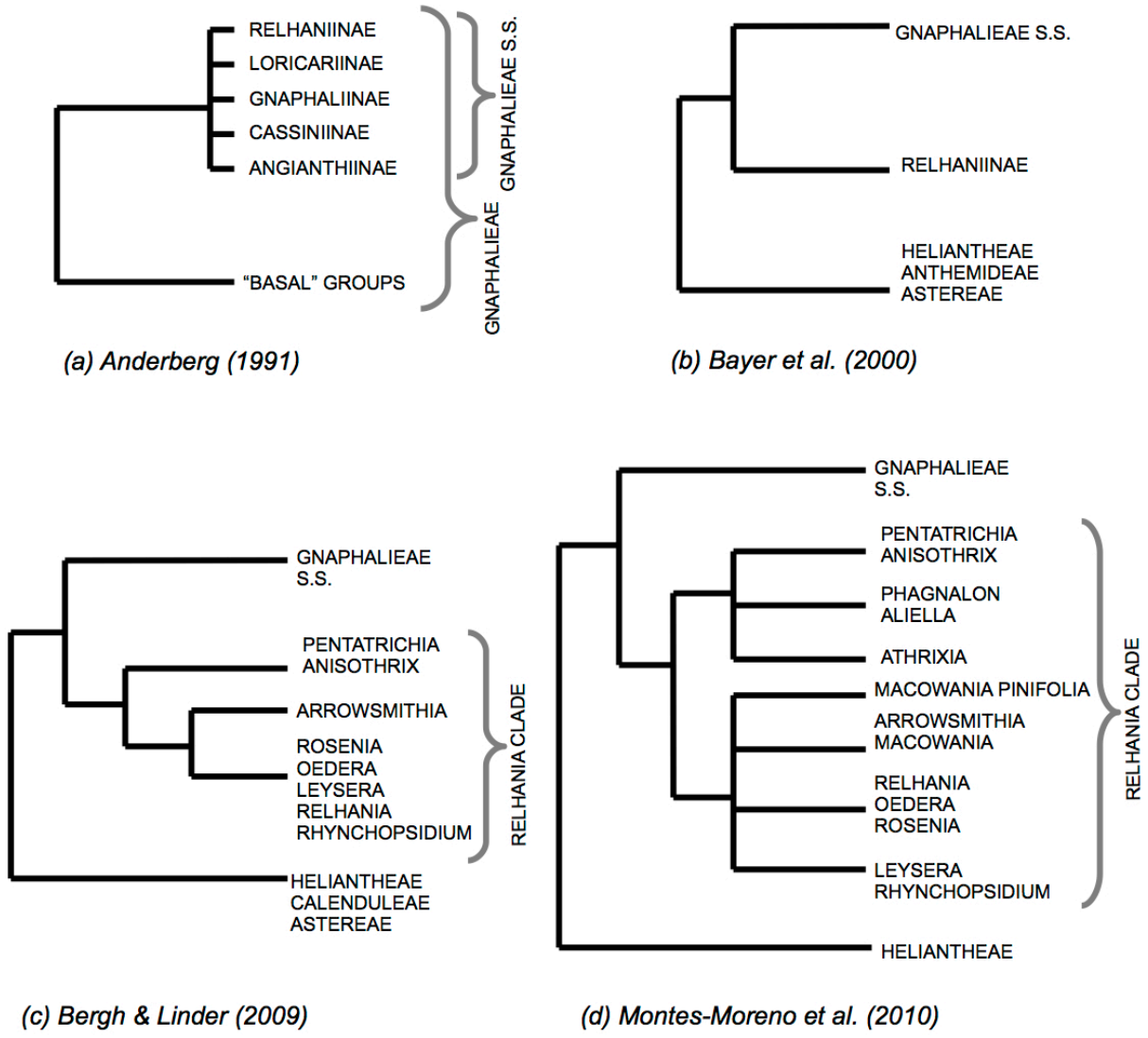



**Fig. 2.1 a – d. Cladograms illustrating the progression in understanding of the relationships between and within the *Relhania* clade and Gnaphalieae *sensu stricto* (s.s.).**

The available molecular phylogenetic studies suggest that there are two principal relhanioid lineages. The first contains the genera *Athrixia, Pentatrichia* and *Phagnalon* (Montes-Moreno et al.

2010; Klaassen & Bergh 2012) and the second, the remaining members of the *Relhania* clade (Anderberg & Bremer 1991; Bergh & Linder 2009; Montes-Moreno et al. 2010; Klaassen & Bergh 2012:*"Oedera*" clade). For the purposes of this thesis, the terms "Athrixiinae" and "Oederinae" will be used to refer to these two principal lineages, respectively. These terms are used informally here and are not intended to denote new subtribal definitions. Furthermore, the term "Gnaphalieae *sensu stricto*" is used to refer to Gnaphalieae *sans* the *Relhania* clade, while "Gnaphalieae" is used to refer to this latter group plus the *Relhania* clade.

The genera in Athrixiinae comprise several rather distinct lineages. All members of this lineage possess serrate to dentate leaves (Fig. 2.2 b, d, f), though in some cases this may be minutely so (e.g. in *Athrixia,* Fig. 2.2 c). *Athrixia* is diagnosed by its distinctively awned capitular involucral bracts (Fig. 2.2 a) and molecular studies identify this genus as monophyletic, though on the basis of a small species sample (Klaassen & Bergh 2012; Montes-Moreno et al. 2010). The closely-related Cape genus *Anisothrix* was recently transferred to the Namibian-centered *Pentatrichia* (Klaassen & Bergh 2012) based on a parsimony analysis of one plastid and two nuclear regions, as well as morphological evidence. *Pentatrichia* members possess either discoid (Fig. 2.2 d) or radiate (Fig. 2.2 b, f) capitula, the latter character being one that they share with *Athrixia.* Using only *Athrixia* as an outgroup, Klaassen & Bergh (2012) identify a monophyletic *Pentatrichia,* though limited sampling and poor resolution prohibit a robust conclusion. The closely-related circum-Mediterranean genus *Phagnalon* (Montes-Moreno et al. 2010; Montes-Moreno et al. 2013) is easily identified by its disciform capitulum, sharing many characteristics with *Pentatrichia*, including long caudate anthers, waxy cushions on the outsides of the corolla lobes, no myxogenic hairs on the achene surface and acute sweeping hairs arranged apically on the stigmatic surface. Previously, members of the genus *Phagnalon* comprised the genera *Phagnalon* and *Aliella.* Montes-Moreno et al. (2010) had difficulty resolving a monophyletic *Phagnalon* and *Aliella* owing to the incongruent placement of two taxa, *Aliella iminouakenis* and *Phagnalon latifolium,* and cited ancient

hybridisation to account for this incongruence. Analyses rerun without these two taxa resolved both genera as monophyletic (Montes-Moreno et al. 2010). A more recent molecular phylogenetic study, with the addition of morphology, transferred *Aliella* to *Phagnalon* (Montes-Moreno et al. 2013). One monotypic genus, *Alatoseta tenuis,* is unplaced in Gnaphalieae but shares some morphological features with *Athrixia,* including pungent points of the involucral bracts similar to the awns of *Athrixia*, pink-purple rays and slender leaves (Fig. 2.2 e: Compton 1931).

Generic boundaries in Oederinae are less clear, attracting extensive discussion (e.g. Anderberg 1991; Anderberg & Bremer 1991; Bremer 1976, 1976b, 1978; Hilliard & Burtt 1985; Kroner 1980). The Afrotemperate endemic, *Macowania,* is easily recognised by its synapomorphically revolute leaves with a prominent raised abaxial midrib which gives the leaf its characteristically grooved appearance (Fig. 4.2 in Chapter Four). Molecular analyses (Bergh & Linder 2009; Montes-Moreno et al. 2012) indicate that it is more closely allied to Oederinae than Athrixiinae, though this has not been rigorously tested. The monotypic *Arrowsmithia* shares revolute leaves (though they are only marginally so), near-identical capitulum structure and stem cylinder anatomy with *Macowania* (Anderberg 1991) and its geographic distribution also coincides with that of *Macowania*, leading some workers (Fig. 2.2 g: Kroner 1980; Hilliard & Burtt 1985; Anderberg 1991) to suggest that the two genera are closely-related. The anomalous *Macowania pinifolia* does not possess the characteristics typical of the genus, but does not fit into any other recognised genus. Instead, it possesses rays which are white above and mauve below and distinct ungrooved, needle-shaped leaves and a long peduncle (Fig. 4.2 in Chapter Four). This has led to some discussion of its generic position (Anderberg 1991; Kroner 1980), with some believing that it is more closely-related to *Athrixia* than *Macowania* (Hilliard & Burtt 1985).


**Fig. 2. 2. Morphological plate illustrating the diversity of the *Relhania* clade.**

**a:** inflorescence of *Athrixia elata* **b:** inflorescence of *Pentatrichia alata* **c:** needle-shaped leaves of *A. elata* **d:** inflorescence of *P. kuntzei* **e:** *Alatoseta tenuis* **f:** *P. alata* **g:** *Arrowsmithia styphelioides* **h:** *Oedera capensis* showing leafy involucres and compound head (in insert) **i:** *Relhania speciosa* **j:** *Oedera sedifolia* **k:** spathulate, felty leaves of *R. rotundifolia* **l:** *Comborhiza virgata* **m:** brown, papery bracts of *Rosenia glandulosa* **n:** *Antithrixia flavicoma* **o:** *Rhynchopsidium sessiliflorum*. Images obtained from the *Relhania* clade digital key (Busch & Bergh 2013).

*Oedera* has the most species of all genera in Oederinae, possessing a diversity of morphological forms and occupying many environments (Fig. 2.2 h, j). Earlier transferred from Anthemideae to Gnaphalieae by Anderberg & Källersjö (1988), the genus initially contained only those members with an unusual secondarily compound head and leafy pseudo-involucres (these members will hereafter be referred to as "true-*Oedera"* Fig. 2.2 h); they also share the unique character of small marginal teeth on the leaves which have been shown to be derived from hairs rather than from the leaf margin (Anderberg 1991). A cladistic morphological analysis (Anderberg & Bremer 1991) found that *Relhania,* currently the second largest genus in Oederinae, was not monophyletic relative to *Oedera* and several members of *Relhania* were transferred to *Oedera* (e.g. j in Fig. 2.2)*,* greatly weakening the diagnosability of *Oedera*. Though some authors believe *Rosenia* (Fig. 2.2 m) to be indistinguishable from *Relhania* (Phillips 1951), others (Anderberg & Bremer 1991; Bremer 1976) have argued for the distinction of these genera on the strength of the presence of pappus bristles in *Rosenia* (*Relhania* generally has none), as well as their vicariant distributions. Two species recently transferred out of *Relhania* now form the genus *Comborhiza* (Anderberg & Bremer 1991)*,* these species being united by their putatively synapomorphous storage tuber. Two monotypic genera, *Oreoleysera* and *Antithrixia* (Fig. 2.2 n), seemingly bear no obvious close relatives (Anderberg & Bremer 1991) and are somewhat anomalous in the group. A group of genera with several species possessing an annual or otherwise short-lived life-history (*Leysera, Rhynchopsidium, Nestlera*) is believed to form a clade (Fig. 2.2 o: Anderberg & Bremer 1991) though its relationship to other members of Oederinae has not been tested molecularly by inclusion of more than a few species. No molecular analysis of the *Relhania* clade has included more than a few representatives of *Oedera* or *Relhania*.

Molecular phylogenies of the recent past (e.g. Bayer et al. 2000; Bergh & Linder 2009; Smissen et al. 2011; Ward et al. 2009) have signalled the existence of the *Relhania* clade through the use of a few placeholder species for each of the genera, though the generic and species-level relationships in

the clade have not been rigorously assessed. Thus, a comprehensive, species-level molecular phylogeny is currently lacking for the *Relhania* clade. Addressing this gap, the aim of this chapter is to assemble a rigorous phylogenetic hypothesis for the *Relhania* clade, including sufficient outgroup taxa to ensure correct rooting of the tree and test the generic circumscription and membership of the *Relhania* clade, using this to evaluate and, where necessary, rearrange generic boundaries. The phylogenetic hypothesis for the *Relhania* clade will be based on multiple nuclear and plastid DNA markers, with node support examined via parsimony bootstrap and Bayesian methods of phylogeny reconstruction. While DNA sequence data provides huge phylogenetic power, adaptations primarily impact the phenotype, thus morphology will be used as an additional source of diagnostic characters, these being identified using published literature.

## Materials and Methods

### Sampling

Plant material was collected throughout South Africa (Table 2.1: shown at the end of this chapter). Where silica-dried material could not be obtained, herbarium material was sampled from BOL, K, NBG, NU and PRE. For the genus *Phagnalon,* sequences lodged on Genbank were used. The ingroup consisted of 86 species, most of which are represented by multiple (up to four) accessions. Sampling aimed to capture the entire geographic range of the species wherever possible. Five species were not sampled (*Athrixia debilis, A. nyassana, Relhania decussata, Oedera foveolata, O. laevis*); these taxa do not occur in South Africa or else are known only from very few specimens, and attempts to locate them in the field and/or extract DNA were unsuccessful. Since the taxonomy of the Mediterranean-centred genus *Phagnalon* has already been recently addressed (Montes-Moreno et al. 2010, 2013), only a subset of the species of *Phagnalon* were included; this subset included the type species and several representatives from across the geographic range. Outgroup taxa from within Gnaphalieae included single representatives of *Ifloga* (representing the *Ifloga*

clade of Bergh et al. 2011), *Dolichothrix, Amphiglossa* and *Stoebe* (representing the *Stoebe* clade of Bergh & Linder 2009), *Gamochaeta* and *Lasiopogon* (representing the crown radiation of Gnaphalieae). Non-gnaphalioid outgroup taxa included *Senecio* (Senecioneae), *Felicia, Printzia* (Astereae), *Cotula, Osmitopsis* (Anthemideae), *Dimorphotheca, Garuleum* and *Calendula* (Calenduleae). *Philyrophyllum* is thought to be closely-related to, if not a member of, Gnaphalieae (Anderberg 1991; Montes-Moreno et al. 2010) and since its taxonomic position has not been recovered, three accessions from each of the two species of *Philyrophyllum* were also included.

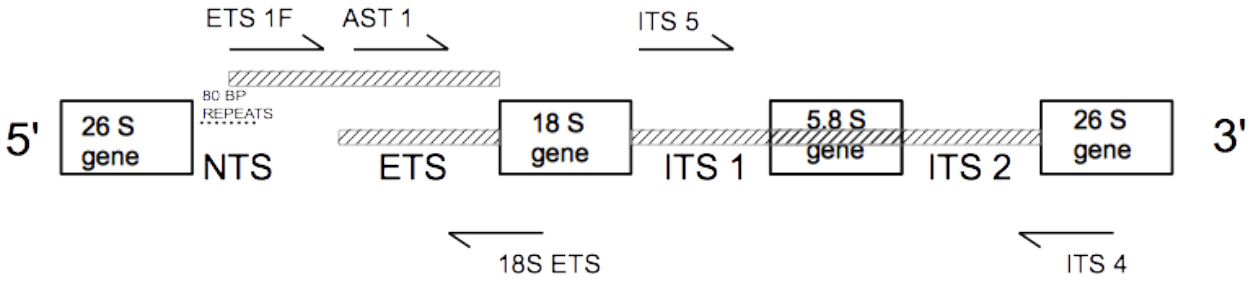



**Fig. 2.3. Simplified illustration indicating the nuclear markers used (not to scale). Primers are indicated by one-bladed arrows (forward primers at top, reverse primers below).** The hatched boxes represent the amplified regions. NTS = non-transcribed spacer, ETS=external transcribed spacer (to ribosomal unit), ITS=internal transcribed spacer (to ribosomal unit). Adapted from Markos & Baldwin (2001) and Starr et al. (2003).

## 2.2. DNA isolation and sequencing

Total genomic DNA was isolated from silica-dried, field-sampled material using the CTAB extraction protocol of Doyle & Doyle (1987) modified according to Gavel & Jarrett (1991), while the Qiagen DNeasy plant extraction-kit (Qiagen Sciences, Valencia, California, U.S.A.) was used for herbarium material. Two nuclear and two plastid regions with proven phylogenetic utility in Gnaphalieae were utilised (Bayer et al. 2002; Bergh & Linder 2009; Galbany-Casals et al. 2004). The external transcribed spacer (ETS) of nuclear ribosomal DNA (Fig. 2.3) was amplified using the reverse primer 18S-ETS (Baldwin & Markos 1998) and the forward primers AST-1 (Markos & Baldwin 2001) and ETS1-F (Starr et al. 2003) while the associated ITS1 and ITS2 introns and the

intervening 5.8S ribosomal gene were amplified as a unit using the ITS5 and ITS4 primers of White et al. (1990; Fig. 2.3). For the chloroplast genome, the *trnT-trnL* region was amplified using the primers 'Tab A' and 'Tab B' (also named "trna" and "trnb") of Taberlet et al. (1991) and the entire *trnL-trnF* region including the *trnL* intron and *trnL-F* spacer was amplified with the 'Tab C' and 'Tab F' primers (Taberlet et al. 1991), and treated as a single region in the analyses. The conservative *trnL-trnF* region is informative at higher taxonomic levels (Gielly & Taberlet 1994) while the faster-evolving ITS region complements this by providing more resolution at the genus and species levels (Baldwin et al. 1995; Galbany-Casals et al. 2004). ETS has also been shown to be variable and informative in Asteraceae (Linder et al. 2000) and so was included to improve resolution and support. Table 2.2 provides a description of the primer sequences used for all regions.

**Table 2.2 Descriptions of the primers used for plastid and nuclear regions.**

| Genome & primer name | Sequence | Reference |
|---|---|---|
| plastid trnT$^{UGU}$F (Tab A) | 5' CAT TAC AAA TGC GAT GCT CT 3' | Taberlet et al. 1991 |
| plastid trnL$^{UAA}$R (Tab B) | 5' TCT ACC GAT TTC GCC ATA TC 3' | Taberlet et al. 1991 |
| plastid trnL$^{UAA}$F (Tab C) | 5' CGA AAT CGG TAG ACG CTA CG 3' | Taberlet et al. 1991 |
| plastid trnF$^{GAA}$ (Tab F) | 5' ATT TGA ACT GGT GAC ACG AG 3' | Taberlet et al. 1991 |
| nuclear ITS 4 | 5' TCC TCC GCT TAT TGA TAT GC 3' | White et al. 1990 |
| nuclear ITS 5 | 5' GGA AGT AAA AGT CGT AAC AAG G 3' | White et al. 1990 |
| nuclear 18S ETS | 5' ACT TAC ACA TGC ATG GCT TAA TCT 3' | Baldwin & Markos 1998 |
| nuclear AST-1 | 5' CGT AAA GGT GCA TGA GTG GTG T 3' | Markos & Baldwin 2001 |
| nuclear 1F | 5' CTG TGG CGT CGC GCA TGA GTT G 3' | Starr et al. 2003 |

PCR amplification was performed in an Applied Biosystems 2720 thermal cycler (Applied Biosystems CA, USA) with the following thermal profile: initial denaturation of two minutes at 94°C; 35 cycles consisting of 94°C for 45 sec, 52°C for 45 sec (annealing) and 72°C for two min (extension); and a final extension step of 72°C for eight min. Reaction mixtures consisted of 12.8 µl nuclease-free $H_2O$, 2.5 µl of 10x buffer (Kapa Biosystems Inc., MA, USA), 1.5 µl of 25 µM $MgCl_2$,

1 µl dNTP mix at 0.2 µM each dNTP, 0.5µl DMSO, 1.25 µl of each primer at 10 µM, 0.2 µl of Taq DNA polymerase (Kapa Biosystems Inc., MA, USA) and 4 µl of template DNA at various dilutions. Successfully amplified target DNA was cleaned and sequenced either by Macrogen Inc (www.macrogen.com/eng/sequencing/sequence_main.jsp) or by the Central Analytical Facility at Stellenbosch University (South Africa) using BigDye terminator cycling. The products were purified using ethanol precipitation and visualised on an ABI Automated Sequencer 3730XL (Life Technologies Corporation, Carlsbad, California, USA.).

### 2.3. Sequence assembly and alignment

Chromatograms were examined and corrected where necessary, and contigs assembled using Geneious Pro V. 5.4.4 (Biomatters Ltd. 2011). Sequences were aligned manually in Bioedit V. 7.1.3.0 (Hall 1999) by matching conserved sections of each region and then working from those into the less-conserved regions. Sections that could not be objectively aligned across all taxa were noted and carefully examined for evidence of homologous sites. For sections that did not have an objective alignment solution, if the alignment problem affected only a few (e.g. outgroup) taxa, the relevant sequence section for these taxa was replaced by '?' (missing data). If a section was tenuous to align across all or most taxa, the section was excluded from phylogenetic analysis by setting up a character exclusion set. The final alignment was checked independently by three people to confirm homology assessment.

### 2.4. Indel coding

In the preliminary stages of the phylogenetic analyses gaps were coded as binary data using the "simple indel coding" method of Simmons & Ochoterena (2000) using the GapCoder software (Young 2003). However, the resulting trees exhibited no increase in resolution and in some instances, the gap characters appeared to add 'noise' i.e. random phylogenetic signal to the data. Thus the inclusion of gaps into analyses was decided against. Another factor influencing the

decision to exclude gaps is that the parameterisation of gaps into the dating analyses and biogeographic models is a complex (and possibly theoretically unsound) procedure. Though it is possible to include gaps in such analyses, it is not possible to model a clock-like evolutionary process for indels. In all analyses, indels were coded as missing data.

## Molecular phylogenetic analyses

### Choice of species accessions

While several species accessions were originally sequenced, some of these yielded poor PCR results and/or sequences which were either too short or difficult to interpret for some markers, and thus could not be used. This resulted in species accessions with missing data for some of the sampled loci. Although the inclusion of such incompletely sampled taxa might lead to consensus trees being poorly resolved (Huelsenbeck 1991; Kearney 2002), there is some evidence to show that the positions of even highly incomplete taxa may be accurately resolved (Givnish & Sytsma 1997; Wiens 2006), the number and quality of characters being most influential. Due to the generally poor resolution of plastid data at deeper nodes (for example see Taberlet et al. 1991; Shaw et al. 2005), accessions represented only by plastid sequences were removed from the matrix. Similarly, the majority of accessions represented by a single nuclear region (and no other marker) were excluded. Only in the case of species represented by a single accession, or whose DNA had proved difficult to obtain (e.g. for species with hard-to-reach distributions in other countries, such as the under-collected *Macowania ericifolia* from the high mountains of Ethiopia and Eritrea), were single nuclear marker accessions retained. For example, the under-collected and rare *Comborhiza longipes* and *Oreoleysera montana,* which are represented by single species accessions, were successfully sequenced only for ITS. To assess the degree to which the inclusion of these taxa influenced support values, separate Bayesian and parsimony analyses were conducted with these two taxa trimmed from the matrix. Since the resulting trees did not show an improvement in support values, these taxa were retained in the final analyses. Successfully sequenced regions for accessions

included in the final analyses are indicated in Table 2.1.

### Assessment of conflict

Phylogenetic conflict was assessed using both parsimony bootstrap percentages (BS) and Bayesian inference posterior probabilities (PP) as described below. Bootstrapping is considered a conservative measure of phylogenetic accuracy (Hillis & Bull 2009) and, as such, is a metric unlikely to result in spurious conflict. To check for topological incongruence, the four DNA regions (ETS, ITS, *trnL-F, trnT-L*) were first analysed individually, and support evaluated using both parsimony bootstrap and Bayesian inference. Since no supported conflict was found, the individual datasets were concatenated to form a combined nuclear (ETS & ITS) and a combined plastid (*trnL-F* & *trnT-L*) dataset. For parsimony analyses, only reciprocally conflicting nodes with BS ≥75% were considered as supported, while for Bayesian inference analyses, PP ≥0.95 was considered supported. Again, no supported conflict was found and so a combined dataset comprising all regions was analysed as described below.

### Parsimony bootstrap phylogenetic analysis

Parsimony searches were executed in PAUP V. 4.0 (Swofford 2002). One thousand bootstrap replicates were performed using only parsimony-informative sites, with tree bisection-reconnection (TBR) branch swapping on 100 random-addition trees per bootstrap replicate with multiple trees saved per random-addition replicate, with the Maxtrees setting at 500. Trees were rooted on *Senecio vulgaris,* which is most likely to be the most distantly related outgroup genus (Panero & Funk 2008).

### Bayesian inference of phylogeny

Bayesian inference was performed using MrBayes V. 3.2 (Huelsenbeck & Ronquist 2001). A mixed model approach was employed in which substitution model parameters were estimated separately

for each of the four DNA data partitions. For this purpose, MrModeltest V. 2.2 (Nylander 2004) was used to determine the model optimality under the AIC criterion (Akaike 1973, 1994). This identified the GTR + G model as optimal for ITS, the GTR + I + G model for ETS and the GTR + G model for each of the two plastid regions. For the individual locus analyses, the settings were identical to the combined analyses (described below), apart from the number of generations which was set to $6 \times 10^6$. Parameter estimation was achieved using a Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) sampling procedure (Gilks et al. 1996; Yang et al. 1997) with analyses starting with a different random tree and running for $10^7$ generations with parameters being sampled every 1,000 generations. The chain heating parameter was set at 0.3 and apart from the model settings, the default settings were retained. Two concurrent runs were performed in each analysis, with the analysis being repeated three times, resulting in a total of six independent runs (i.e. 6 x $10^7$).

### Examination of convergence

Convergence of individual MCMCMC runs was assessed by examining the average standard deviation of split frequencies from the MrBayes output to determine when this value dropped below 0.01 (see MrBayes manual). The tree topologies and posterior probability support values from the six independent runs were also compared to check whether the runs converged on the same topology. Convergence was further tested using Tracer V. 1.3 (Drummond & Rambaut 2007) to examine parameter estimates, ESS scores and likelihood traces. After examining convergence, the first 10% of samples from each run were discarded as burn-in, the remaining parameters being summarised on the 50% majority-rule consensus tree obtained from all runs, using TreeAnnotator V. 1.6.2 (Drummond & Rambaut 2007).

### Morphological analyses

Since species-level phylogenetic relationships within Oederinae were poorly resolved by molecular

DNA analysis (see results), a matrix of morphological characters was used to supplement phylogenetic inference within this group. For the morphological character analysis, a 46-character matrix was used consisting of characters describing a range of morphological attributes, from growth form to cypsela anatomy, compiled by Anderberg & Bremer (1991) on the basis of earlier taxonomic revisions (e.g. Anderberg 1991; Bremer 1976, 1976b, 1978). With the exception of species of *Macowania* and *Arrowsmithia*, the under-collected *O. epaleacea* and an undescribed compound-headed species of *Oedera* (the latter two unknown to Anderberg & Bremer 1991), all members of Oederinae were included in this matrix. The morphological data were used in two ways. First, the morphological matrix was subjected to parsimony analysis in PAUP V. 4.0 with the settings identical to those outlined above. The resulting trees were rooted on *Leysera* + *Rhynchopsidium* + *Nestlera.* Second, parsimony analysis was conducted on the concatenated nucleotide and morphological data with the molecular matrix edited to include only those taxa represented by Anderberg & Bremer (1991). Where species are represented by multiple accessions in the DNA matrix, a single representative accession was chosen based on data completeness for all four gene regions.

# Results

### Molecular phylogenetic analyses

Small (<20 base pair [BP]), difficult to align sections were identified in the nuclear alignments. In the data matrices these were replaced with "?" (missing data). The relevant sections were: a 19 BP segment in the middle of ITS in *Calendula,* a 17 BP section (also in the middle but not the same section) in ITS in *Printzia,* and one section ranging from 6 to 9 BP near the 3' end of ETS in four outgroup taxa. Apart from this, the nuclear ITS region did not have any ambiguous alignment segments. A 314 base pair (BP) insertion in *Calendula officinalis* in *trnL-F* was excluded from analyses. The 3' region of ETS, amplified with the AST-1 forward primer, was easily aligned across

all taxa, including outgroups. Aligning the 5' end of the nuclear ETS region corresponding to the ETS IF forward primer (see Fig. 2.3), however, proved to be more challenging. A section of about 680 nucleotides at the 5' end of the amplified region was highly variable and, although it could be unambiguously aligned between certain genera within the *Relhania* clade, it could not be objectively aligned between or within all genera sequenced for this region. In addition, there was a significant amount of missing data for this 5' end of ETS, because many accessions were amplified using AST1 rather than the 1F primer. For these reasons, the variable 5' end of ETS was included only for *Comborhiza, Oedera, Relhania* and *Rosenia,* being coded as missing data for all other taxa.

The plastid *trnT-L* matrix consisted of 123 accessions and 787 aligned nucleotides, 19% of which are parsimony-informative (PI), and the plastid *trnL-F* matrix consisted of 79 accessions and 1,324 aligned nucleotides (11% PI). The two separate plastid regions yielded poorly-resolved but congruent trees, justifying their combination to form a matrix consisting of 150 accessions and 1,797 characters (16% PI). The nuclear ETS matrix consisted of 152 taxa and 1,147 aligned sites (54% PI). The ITS matrix consisted of 172 taxa and 723 aligned sites (50% PI). Analysis of these two nuclear regions independently produced well-resolved and completely congruent trees. These were combined to form a single nuclear matrix consisting of 182 accessions and 1,871 aligned nucleotides (52% PI). The trees resulting from the separate plastid and nuclear datasets are shown in Figs. 2.4 a & b. Given that there was no supported conflict between the nuclear and plastid topologies, the two regions were combined, forming a single matrix consisting of 179 accessions and 3,981 aligned nucleotides (29% PI). The resulting combined Bayesian tree is presented in Figs. 2.5 a & b.

The analyses yielded generally well-resolved trees with no incongruence between the different molecular markers. Overall, the trees presented here corroborate the higher-level relationships resolved by recent molecular studies of Gnaphalieae and relatives (e.g. Bayer et al. 2000; Bergh &

Linder 2009; Ward et al. 2000; Panero & Funk 2008). The plastid tree (Fig. 2.4a) recovers *Philyrophyllum* as monophyletic and places it outside of Gnaphalieae (BS=94, PP=1.0), and resolves Gnaphalieae *sensu stricto* as monophyletic (BS<75, PP=1.0). The *Relhania* clade is also monophyletic (BS=97, PP=1.0), as are Athrixiinae (Node A: BS=92, PP=1.0) and Oederinae (Node B: BS=97, PP=1.0). Within Athrixiinae, *Phagnalon* is monophyletic (Node D: BS=86, PP=1.0). Relationships within Oederinae are largely unresolved, though a clade consisting of all short-lived annual and biennial genera is recovered (Node G: BS<75, PP=1.0: the "short-lived" clade). Species of "true-*Oedera*", with the compound heads, group together (Node I: BS<75, PP=0.99). In the nuclear tree (Fig. 2.4b), Gnaphalieae is recovered as monophyletic (BS=97, PP=1.0) with the monophyletic *Philyrophyllum* (BS=100, PP=1.0) excluded. Gnaphalieae *sensu stricto* is monophyletic (BS<75, PP=0.95). The *Relhania* clade is monophyletic (BS=100, PP=1.0) as are Athrixiinae (Node A: BS=75, PP=1.0) and Oederinae (Node B: BS=100, PP=1.0). Within Athrixiinae, *Alatoseta tenuis* is recovered as sister (BS<75, PP=0.97) to a monophyletic *Athrixia* (Node C: BS=100, PP=1.0), and *Phagnalon* is monophyletic (Node D: BS=85, PP=1.0) and sister (BS=100, PP=1.0) to *Pentatrichia* which is not supported as monophyletic by bootstrap or Bayesian analysis (Node E: BS<75, PP<0.95). In Oederinae, *Macowania* and *Arrowsmithia* group together (Node F: BS=84, PP=1.0) and are sister to the remaining members of Oederinae. The "short-lived" clade is monophyletic (Node G: BS=100, PP=1.0), and is sister to the remaining members of Oederinae, henceforth named "Oederinae *sensu stricto*" (Node H: BS=100, PP=1.0). Within Oederinae *sensu stricto*, several subclades are well-supported including the "true-*Oedera*" clade (Node I: BS=92, PP=1.0), a group consisting of members of *Antithrixia, Comborhiza, Oedera, Relhania* and *Rosenia* (Node J: BS<75, PP=1.0), henceforth termed the "widespread" group, and a clade of members of *Oedera* (termed the "*squarrosa*" clade: Node L: BS=96, PP=1.0). Relationships amongst these subclades and some of the species-level relationships are unresolved.


**Fig. 2.4a. Plastid (trnT-L & trn L-F) Bayesian 50% majority-rule consensus tree**

Node support is indicated above (posterior probability) and below (bootstrap) branches. Only PP values ≥ 0.95 and BS values ≥ 75% are annotated. Nodes A – L correspond to nodes of interest discussed in the text.

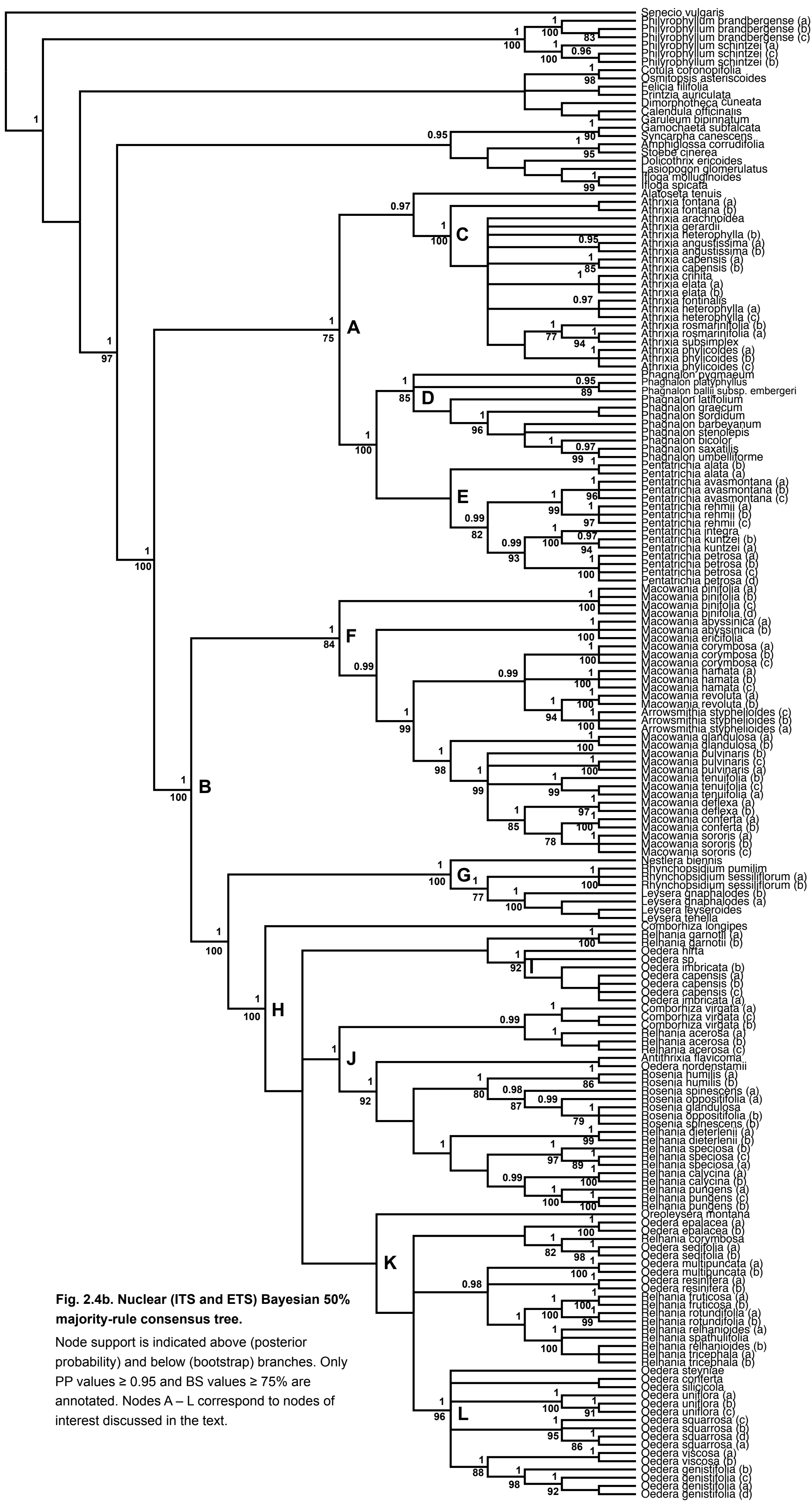



**Fig. 2.4b. Nuclear (ITS and ETS) Bayesian 50% majority-rule consensus tree.**

Node support is indicated above (posterior probability) and below (bootstrap) branches. Only PP values ≥ 0.95 and BS values ≥ 75% are annotated. Nodes A – L correspond to nodes of interest discussed in the text.

The combined tree closely matches the relationships found in the nuclear tree wherein nodes are well-supported. In the combined analyses (Figs. 2.5a & b), Gnaphalieae group together (BS=99, PP=1.0). The most-closely related outgroup in the consensus tree is Astereae, but this relationship is unsupported (BS<75, PP<0.95). Astereae group together (BS<75, PP=1.0), as does Anthemideae (monophyletic with BS<75, PP=0.99) which is sister (BS<75, PP=0.97) to Calenduleae (forming a group with BS<75, PP=1.0). *Philyrophyllum* is recovered as monophyletic (BS=100, PP=1.0) but is not found to be a member of Gnaphalieae; rather, it diverges within the sampled outgroup taxa, indicating that it is a member of the large subfamily Asteroideae, but only distantly related to Gnaphalieae. Gnaphalieae *sensu stricto* (the "crown radiation" of Ward et al. 2009) is recovered as monophyletic (BS=91, PP=1.0). The *Relhania* clade itself is strongly supported as monophyletic (BS=100, PP=1.0), as are both Athrixiinae (Node A: BS=89, PP=1.0) and Oederinae (Node B: BS=100, PP=1.0).

Athrixiinae is divided into two main subclades (Fig. 2.5a), the first containing a monophyletic *Athrixia* (Node C: BS=100, PP=1.0), sister to *Alatoseta tenuis* (BS<75, PP=0.99). The second clade consists of a monophyletic *Phagnalon* (Node D: BS=98, PP=1.0), sister (BS=100, PP=1.0) to *Pentatrichia* (Node E: BS<75, PP<0.95). The monophyly of *Pentatrichia* lacks posterior probability and bootstrap support.

Oederinae is divided into three subclades; node F (*Macowania* and *Arrowsmithia*), node G: ("short-lived clade") and node H (Oederinae *sensu stricto*). Within Oederinae (Fig. 2.5b), *Arrowsmithia* is embedded within *Macowania,* which is otherwise monophyletic (Node F: BS=77, PP=1.0). The "short-lived" clade is well-supported (Node G: BS=100, PP=1.0) and sister (BS=95, PP=1.0) to Oederinae *sensu stricto*. The latter is also well-supported (Node H: BS=100, PP=1.0), though the relationships of several species within this subclade are poorly resolved. The "true-*Oedera*" clade is recovered as monophyletic (Node I: BS=100, PP=1.0), a result that is supported by all analyses

except that based on *trnT-L*. There is also support for the "widespread" group (Node J: BS=87, PP=1.0). Some species' positions are unresolved, including those of *C. longipes* and *R. garnotii,* but the unresolved placement of *C. longipes* is potentially attributable to the fact that it was sampled only for ITS. *Comborhiza* is non-monophyletic while *Rosenia* is supported as forming a clade (BS=75, PP=1.0). Another supported group comprises the majority of *Oedera* species and Cape-centred *Relhania* species together with *Oreoleysera* (Node K: BS<75, PP=0.98). This is henceforth termed the "Cape" group. Within the "Cape" group, the "*squarrosa"* clade is well-supported (Node L: BS=99, PP=1.0).

Some species are resolved as non-monophyletic. For instance, in Oederinae, one accession of *R. spinescens* is placed as sister to *R. glandulosa* and not to the other accession of *R. spinescens.* Similarly, one accession of *L. gnaphalodes* is resolved as sister to *L. tenella* and not the other accession of *L. gnaphalodes.* Two accessions of *O. imbricata* are also placed differently in "true-*Oedera*". In Athrixiinae, one accession of *A. heterophylla* does not group with the other two accessions of this species. These incongruent placements are likely attributable to incomplete lineage sorting. One sample of *A. rosmarinifolia* groups with *A. subsimplex,* though there was uncertainty in the identification of this specimen, which will need to be re-examined.

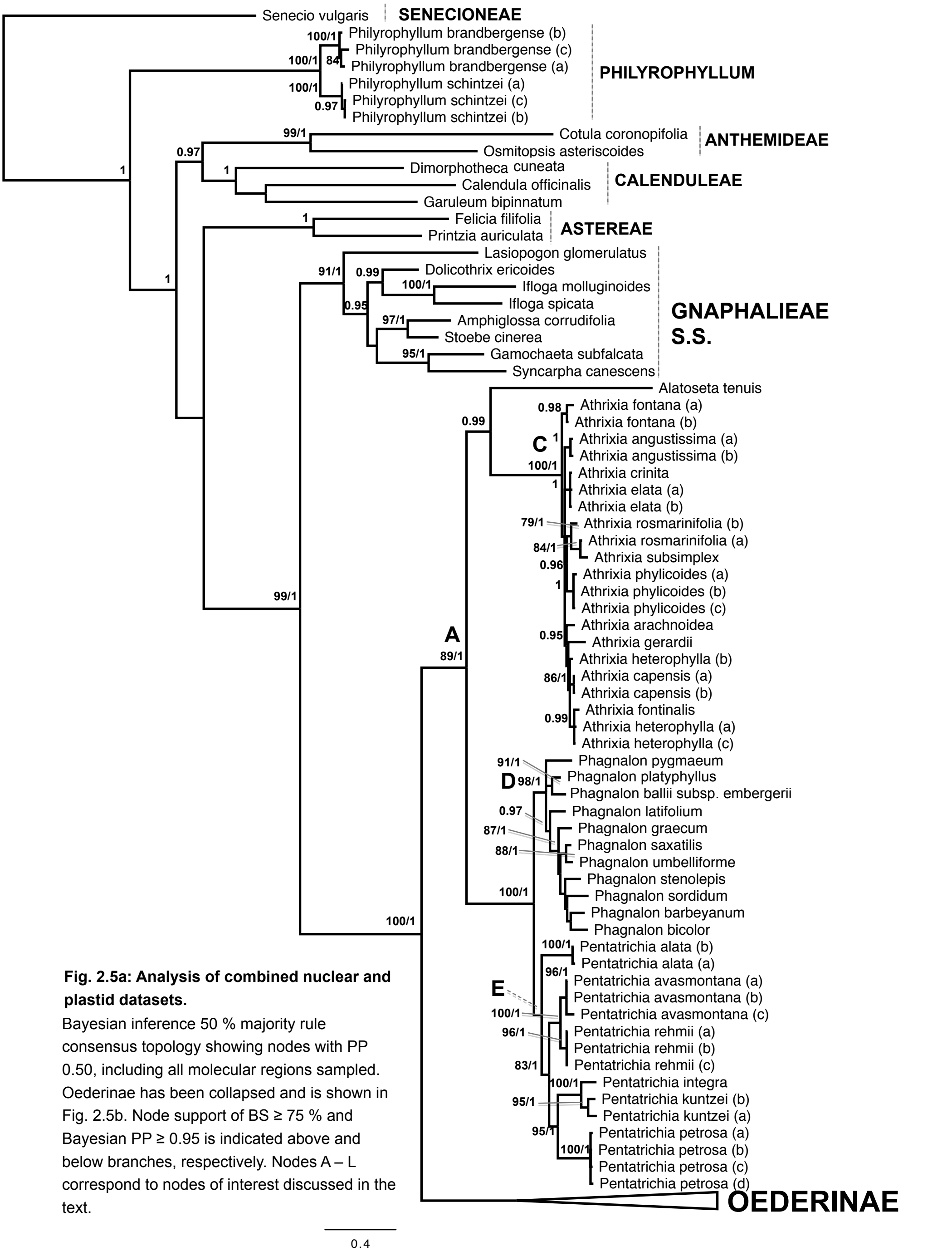



**Fig. 2.5a: Analysis of combined nuclear and plastid datasets.**

Bayesian inference 50 % majority rule consensus topology showing nodes with PP 0.50, including all molecular regions sampled. Oederinae has been collapsed and is shown in Fig. 2.5b. Node support of BS ≥ 75 % and Bayesian PP ≥ 0.95 is indicated above and below branches, respectively. Nodes A – L correspond to nodes of interest discussed in the text.


**Fig. 2.5b: Analysis of combined nuclear and plastid datasets.**

Bayesian inference 50 % majority rule consensus topology showing nodes with PP ≥ 0.50, including all molecular regions sampled. Athrixiinae has been collapsed and is shown in Fig. 2.5a. Node support of BS ≥ 75 % and Bayesian PP ≥ 0.95 is indicated above and below branches, respectively . Nodes A – L correspond to nodes of interest discussed in the text.

## Morphological analyses

The morphological analysis presented here (Fig. 2.6a) is poorly resolved overall but there are some well-supported groupings. For example, species of *Leysera* (BS=96), *Rhynchopsidium* (BS=91) and the "true-*Oedera*" clade (Node: I: BS=99) group together, and there is also support for a clade comprising *R. calycina, R. pungens* and *R. speciosa* ("*speciosa*" clade: BS=100) and all *Rosenia* species plus *Antithrixia* (BS=92). Except for the position of *Antithrixia* these groupings are consistent with those of the molecular analyses.

The combined DNA and morphology tree (Fig. 2.6b) reveals several groupings which are consistent with the morphology-only and molecular-only analyses, including the "true-*Oedera*" clade (Node I: BS=100), *Rosenia* (BS=79), the "*speciosa*" clade (BS=100), and the *"squarrosa"* clade (Node L: BS=99). This tree supports the placement of several taxa within the "widespread" group including *Rosenia, Antithrixia,* the *"speciosa"*clade, *O. nordenstamii and R. dieterlenii* but excludes *C. virgata* and *R. acerosa* (Node Z: BS=89). However, there are also several groupings which are in conflict with the molecular-only analyses. Specifically, two species which are supported as sisters by the molecular analysis (Fig. 2.5a), *O. sedifolia* and *R. corymbosa,* are placed in different clades (marked "X" and "Y" and indicated with arrows in Fig. 2.6b) when morphology is added to the molecular data. On morphological grounds, the placement of these taxa in Fig. 2.6b is more sensible as they share many features with other members of these subclades. For instance, *R. corymbosa* shares eglandular floret tubes, glandular-punctate leaf glands, leaves which are adaxially more densely pubescent than abaxially, involute leaf margins and a naked receptacle with members of its subclade (BS=85) in Fig. 2.6b. Similarly, *O. sedifolia* shares regularly glandular floret tubes, sunken leaf glands, adaxially glabrous leaves, flat leaf margins and a paleate receptacle with other members of this subclade (BS=83) in Fig. 2.6b. This indicates a potential problem with the *R. corymbosa* sequences; this species is represented by only a single accession, and in the nuclear matrix by ITS only. Since it is the nuclear data that resolves species-level relationships, there may

be a problem with the ITS sequence for this accession. The sequence was re-checked and re-amplified, but this produced the same sequence so the species will need to be re-sequenced for nuclear regions, perhaps using a different accession.

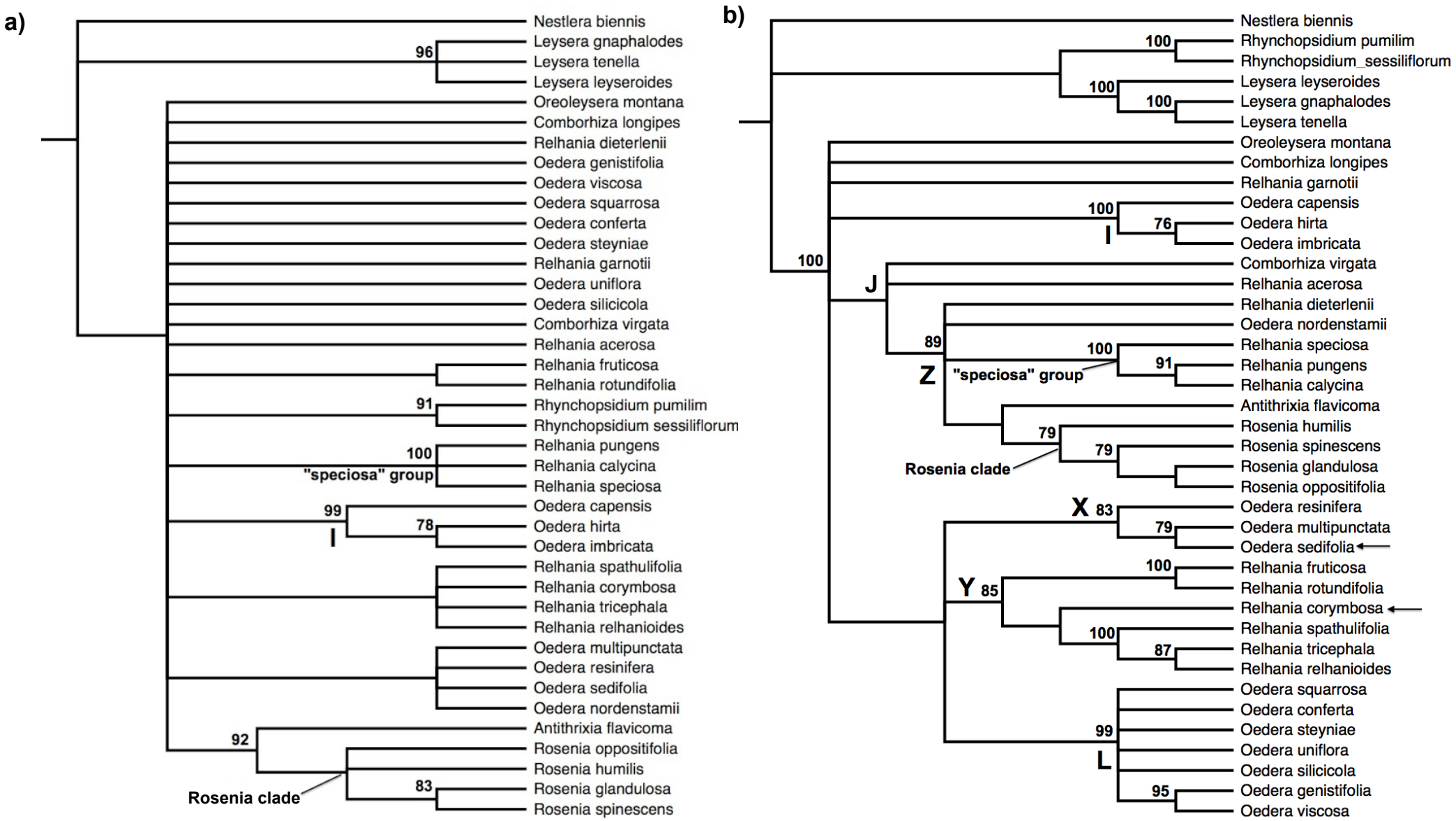



**Fig. 2.6: Parsimony bootstrap 50 % majority-rule consensus trees using the morphological (a) and morphology+DNA (b) datasets.**

Node support ≥ 75% is indicated above branches. The labels X, Y, Z, I, J, L, "speciosa group", "Rosenia clade" correspond to groupings discussed in the text. The arrows indicate the positions of two species discussed in the text.

# Discussion

This chapter presents a rigorously investigated series of phylogenetic hypotheses of relationships in the *Relhania* clade, with fair to good support for most nodes, and support along much of the backbone of the tree. It is the first densely-sampled, species-level molecular phylogeny of all putative members of the *Relhania* clade (86 out of the 91 species, excluding the unsampled members of *Phagnalon*).

Poor resolution at low taxonomic levels is a general feature of plastid-based estimates of phylogenetic relationships in plants (Shaw et al. 2005; Taberlet et al. 1991), and the plastid markers sampled in this study provided few informative sites (16% PI for plastid compared with nuclear 52% PI). However, the combination of *trnT-L* and the *trnL* intron is among the most variable of the combined plastid regions across a range of angiosperm groups (Shaw et al. 2005). The utility of ITS in phylogeny reconstruction has been questioned on account of its multi-copy nature which potentially results in paralogy issues (Álvarez & Wendel 2003). While several phylogenetic studies in Asteraceae utilising ETS and ITS together (e.g. Klaassen & Bergh 2012; Markos & Baldwin 2001; Sanz et al. 2008; Roberts & Urbatsch 2004) have found no significant instances of incongruence (signals of differing evolutionary histories which may be a result of hybridisation, incomplete lineage sorting or incomplete copy homogenisation), others have (e.g. Galbany-Casals et al. 2009; Mitsui et al. 2008). Instances of topological incongruence between nuclear and plastid DNA in Asteraceae are relatively common (e.g. Gnaphalieae: Smissen et al. 2011; *Phagnalon* & *Aliella:* Montes-Moreno et al. 2010; Senecioneae: Pelser et al. 2007; *Melampodium:* Blöch et al. 2009; *Machaeranthia:* Morgan 2003), but luckily, incongruence of this sort was not found in this study. There is minor conflict between the molecular analyses and the morphology + DNA analysis, though conflicting signal between morphology and DNA is also not unusual (e.g. Wiens 2001). This conflict relates only to a single taxon (*R. corymbosa*), and may be the result of a labeling error.

The *Relhania* clade is recovered as sister to the remainder of Gnaphalieae as found by previous studies (Bergh & Linder 2009; Montes-Moreno et al. 2010; Ward et al. 2009). Molecular analysis neither supports nor contradicts the taxonomic inclusion of the *Relhania* clade within Gnaphalieae. Members of the *Relhania* clade possess some features which are unusual in members of Gnaphalieae *sensu stricto*, which might be interpreted as the retention of plesiomorphic features in the early-diverging lineages of a group. These include the presence of ray florets which are rare in Gnaphalieae *sensu stricto*, the attractive function being instead performed by often brightly coloured, modified involucral bracts surrounding the disc florets, giving the capitulum the characteristic papery appearance and the tribe the popular name "everlastings". The possession of papery-textured involucral bracts in Gnaphalieae *sensu stricto* also contrasts with some members of the *Relhania* clade, including *Athrixia, Pentatrichia* and some species of *Phagnalon,* which have leafy or cartilaginous bracts. There are, however, many features shared between the *Relhania* clade and Gnaphalieae *sensu stricto*. These include a pappus of delicate barbellate, scabrid or plumose bristles, woolly leaves, and papery involucral bracts in most members.

*Philyrophyllum* was previously identified as a member of Gnaphalieae (Montes-Moreno et al. 2010) and placed in the informal "basal" group by Anderberg (1991) who considered it to be closely related to *Pentatrichia*. The current analysis indicates no relationship between these two genera, instead demonstrating that *Philyrophyllum* is not a member of the tribe (supported by ETS, ITS and *trnL-F*). This discrepancy is likely attributable to homoplasy in the small sample of morphological characters available to Anderberg (1991). Montes-Moreno et al. (2010) sampled only the *trnL-trnF* region, which did not resolve many relationships, and used only a single species accession of *P. schintzei*. Support for the generic composition of the *Relhania* clade contrasts only with Bayer et al. (2000) wherein *Athrixia* is not found to be a member of the *Relhania* clade. However, their sample of *Athrixia* may have been misidentified (R. Bayer, pers. comm.). Also, whereas Bayer et al. (2000)

placed the monotypic *Oreoleysera* near *Helichrysum*, ITS and morphology support its inclusion in Oederinae *sensu stricto.*

The two informally-named subtribes, Athrixiinae and Oederinae are morphologically distinct and consistently recovered as monophyletic in all analyses. While the relationships of Athrixiinae are largely consistent with previous morphological and molecular analyses (Anderberg 1991; Klaassen & Bergh 2012; Montes-Moreno et al. 2010; Ward et al. 2000), the situation within Oederinae requires further attention. As currently delimited, *Macowania* is non-monophyletic. In their current circumscription, most genera within Oederinae (with the exception of *Rosenia* and the "short-lived" clade) are also non-monophyletic. The key question within Oederinae *sensu stricto* is whether to recognise one monophyletic genus (consisting of 40 sp. including three members not sampled in this study), or several smaller monophyletic genera (e.g. true-*Oedera:* 5 spp., "*squarrosa*"*:* 11 spp., group "X" in Fig. 6b*:* 3 spp., group "Y" in Fig. 6b: 6 spp., *Rosenia:* 4 spp., *"speciosa*": 3 spp, or, the "widespread" group: 12 spp.). One argument for delimiting several smaller genera would be to maximise the amount of information in the taxonomy (Backlund & Bremer 1998), avoiding a "bland", blanket classification which ignores the morphological subtleties of species and genera. The combined DNA + morphology tree resolves some concise groupings, suggesting that it may be possible to separate the group into several genera. However, if one were to recognise several groups based on the current molecular and morphological evidence, the issue remains about how to deal with taxa whose positions are unresolved by most analyses, many of them mono- and ditypic, and which bear no obvious morphological affinity to any particular genus in the group based on an evaluation of the literature available. These include *Antithrixia, Comborhiza, Oedera nordenstamii, Oreoleysera, Relhania acerosa* and *R. garnotii*.

*Athrixia* is recovered as unambiguously monophyletic, supporting previous findings in this regard (e.g. Klaassen & Bergh 2012; Montes-Moreno et al. 2010; Ward et al. 2009). Though 12 of the 14

species of *Athrixia* were sampled, the two unsampled species share the morphological synapomorphies of the clade (e.g. slender leaves, awned involucres) and closely resemble other members of the genus (Kroner 1980). *Alatoseta tenuis,* a monotypic species whose phylogenetic position was formerly unknown, is well-supported as a member of Athrixiinae, being sister to *Athrixia.* Despite sharing many morphological synapomorphies with *Athrixia* (for example, pink-purple ray florets, similar awn-like structures on the involucre, slender leaves), *Alatoseta* has several unusual features, most notably, annuality and a peculiar winged pappus, supporting the decision to maintain it in a separate, monotypic genus.

All analyses resolve a *Pentatrichia* clade and, while there is no clear phylogenetic support for the inclusion of *P. alata* in *Pentatrichia,* there is also no clear support for its exclusion. A principal components analysis of seven characters (Klaassen et al. 2009) grouped *P. alata* and *P. petrosa* together, identifying them as morphologically similar. Morphologically, *P. alata* is more similar to other members of *Pentatrichia* than any other closely-related genus, supporting its inclusion in the genus. There exists a close relationship between *Phagnalon* and *Pentatrichia,* these genera sharing many morphological traits, including long caudate anthers, waxy cushions on the outside of the corolla lobe, no myxogenic hairs on the achene surface and acute sweeping hairs arranged apically on the stigmatic surface (Montes-Moreno et al. 2010). *Pentatrichia* is, however, distinct from *Phagnalon* in possessing discoid or radiate rather than disciform capitula . *Phagnalon* is supported as monophyletic in all analyses, this being in accordance with the recent findings of Montes-Moreno et al. (2013).

Although *M. pinifolia* differs substantially from the rest of *Macowania* in terms of its morphology (needle-shaped leaves with straight margins, white and mauve ray florets, differences in achene characteristics), the analyses presented here suggest that it is best placed with *Macowania*, in which it is the earliest-diverged element. The defining character of revolute leaf margins, a unique feature

in *Macowania*, is absent in this species. One option might be to place it in its own monotypic genus, which might be sensible on morphological grounds (see Anderberg 1991) and would not conflict with the molecular findings. However, the unnecessary proliferation of genera is also undesirable (Backlund & Bremer 1998). The most parsimonious option is thus to retain *M. pinifolia* in *Macowania* despite some morphological incongruence.

*Macowania* is then monophyletic only with the inclusion of *Arrowsmithia styphelioides.* Together with some morphological features (e.g. somewhat revolute leaves with adaxially tomentose surfaces, near-identical capitulum morphology and stem wood cylinders), this argues for the synonymisation of these genera. Given that the name *Arrowsmithia* (De Candolle 1838) is older than *Macowania* (Oliver 1870)*,* the nomenclatural rule of priority requires all species currently included in *Macowania* to be transferred to *Arrowsmithia,* resulting in 11 new combinations to be addressed in a future manuscript (Bentley et al. in. prep).

On current evidence, the simplest treatment for Oederinae *sensu stricto* is to collapse it into a single genus. Although the resulting genus is difficult to characterise morphologically, the definition of multiple genera using monophyly as a primary grouping principle is even harder to achieve. Despite using several sources of available information including both morphology and DNA and multiple species accessions, no analyses conducted in this study successfully resolved all relationships within this clade and, while the addition of morphological data reveals some sensible groupings, it does not provide a solution for the monophyletic classification of the group in its entirety. A one-genus treatment of the group requires that the oldest name in the group (*Oedera*) be prioritised over the remaining genera (*Antithrixia, Comborhiza, Oreoleysera, Relhania* and *Rosenia*)*,* resulting in 21 name changes. Although *R. decussata* was not included in the analyses presented here, there is little doubt that this species belongs in an expanded *Oedera*: Anderberg & Bremer (1991) concluded that this species is firmly nested within Oederinae *sensu stricto* with close morphological affinity to the

group containing *R. speciosa, R. calycina* and *R. pungens,* based on its conspicuous large capitula and decussate, pungent leaves. Two under-collected species for which material could not be attained, *O. foveolata* and *O. laevis*, are poorly understood but the former is believed to be closely allied to *O. sedifolia* (Bremer 1976), while the latter is clearly a member of the compound-headed clade.

Previously found to form a clade (Anderberg & Bremer 1991), the monophyly of *Leysera, Rhynchopsidium* and *Nestlera* ("short-lived" clade) receives unambiguous support from most analyses. The relative position of this clade in Oederinae (i.e. as sister to Oederinae *sensu stricto*) matches that found in previous studies (e.g. Bayer et al. 2000; Bergh & Linder 2009). Though the genera are distinguished by a few morphological characters (e.g. *Rhynchopsidium* has unique apically coiled cypsela hairs, *Leysera* has a receptacle with long squamae) they all possess reduced base chromosome numbers (*Leysera:* x = 4, *Nestlera* and *Rhynchopsidium:* x = 5, compared with other members of Oederinae which generally have x = 7: Anderberg & Bremer 1991) and annuality/bienniality (with the exception of *L. gnaphalodes* which is facultatively annual). The most coherent taxonomic option, in line with the broader '*Oedera*' concept suggested above, is to recognise this short-lived clade as a single genus. Since *Leysera* is the oldest generic name (Linnaeus 1763), a future manuscript will incorporate *Nestlera* and *Rhynchopsidium* into *Leysera,* resulting in three name changes (Bentley et al. in. prep).

Species in an expanded *Oedera* comprise a morphologically varied group. Nonetheless, they are distinguishable on the basis of leaf and capitulum characteristics. Contrasting with the toothed or serrate leaf margins of Athrixiinae, leaves in the expanded *Oedera* clade are always entire, though in the compound-headed species the presence of marginal hairs gives the the appearance of serration. Also, the leaf margins vary from flat to involute, never being revolute as in *Macowania*. Capitulum morphology is also distinctive, the heads always being radiate, contrary to the condition

in *Pentatrichia* where heads are sometimes discoid and in *Phagnalon* where they are disciform. Florets are mostly yellow but may be orange in a few species, whereas in *Athrixia* and *Alatoseta* the florets are white or pink/purple. The involucral bracts are papery, contrasted with *Athrixia, Pentatrichia* and some species of *Phagnalon* which have cartilaginous bracts. The majority of species (apart from *Rosenia, Oreoleysera* and *Antithrixa*) have a pappus which lacks bristles, whereas the pappus of *Macowania,* the short-lived clade and Athrixiinae are all made up of numerous bristles.

## Conclusion

A molecular phylogenetic analysis of plastid and nuclear DNA data with extensive ingroup and modest outgroup sampling is presented, with no instances of topological incongruence or conflict being found between the molecular data, and only minor conflict between the molecular and morphological datasets. The *Relhania* clade is confirmed as monophyletic and sister to Gnaphalieae, and there is an argument for its inclusion in this tribe based on molecular evidence from several sources, supported by morphology. The taxonomic classification of this group is unlikely to be undertaken by another author in the near future, so a concise classification at this stage is paramount. This study also confirms, and rejects, several hypotheses proposed by previous authors. *Philyrophyllum* is not a member of Gnaphalieae and *Alatoseta*, whose phylogenetic position was previously unknown, belongs in Athrixiinae, being sister to the monophyletic *Athrixia. Macowania pinifolia* is confirmed as a member of *Macowania* despite its unusual morphology. *Macowania* is then monophyletic if synonymised with *Arrowsmithia,* and this clade is then sister to the remaining species of Oederinae. The generic circumscription of Oederinae is particularly challenging as it is a highly diverse group. It consists of two primary well-supported clades: the smaller of the two clades houses all genera that have short-lived species (*Leysera, Rhynchopsidium* and *Nestlera*) and these genera are best collapsed into an expanded *Leysera*. Within Oederinae

*sensu stricto*, some of the taxa, currently assigned to *Antithrixia, Comborhiza, Oedera, Oreoleysera, Relhania, Rosenia*, are recovered in several morphologically distinct clades: "true-*Oedera*", *Rosenia,* "*speciosa*" clade, group "X" and "Y", "*squarrosa*" clade. While these clades may each merit independent generic status, there are several species whose placement is not clear, and despite appealing to both molecular and morphological lines of evidence, division of this clade into monophyletic entities is not a possible solution to entertain currently. For this reason, these six genera are proposed to be included into one generic-level entity, viz. *Oedera sensu* Bentley. The new combinations and taxonomic changes mentioned will not be made here and will rather be published in a series of forthcoming papers.

**Table 2.1. Sampling table of species accessions used, indicating collection information and regions sampled.**

| Species Name/Accession | Collector Information | Locality | Herb | Regions Sampled | | | |
|---|---|---|---|---|---|---|---|
| | | | | Nuclear | | Plastid | |
| | | | | ETS | ITS | *trnTL* | *trnLF* |
| *Alatoseta tenuis* | JC Manning 3187 | Tankwa Karoo | NBG | X | X | - | X |
| *Anithrixia flavicoma* | NG Bergh 2095 | Kamiesberg | NBG | X | X | - | - |
| *Athrixia angustissima (a)* | M Koekemoer 3550 | Sehlabathebe National Park | PRE | X | X | X | - |
| *Athrixia angustissima (b)* | NG Bergh 1499 | Mashai's Nek, Drakensberg | NBG | X | X | - | X |
| *Athrixia arachnoidea* | NG Bergh 2198 | Cathedral Peak Drakensberg | NBG | X | X | X | X |
| *Athrixia capensis (a)* | BA Walton 533 | Overberg | NBG | X | X | - | X |
| *Athrixia capensis (b)* | CREW & H Stummer 3597 | Tygerberg Nature Reserve | NBG | - | X | - | X |
| *Athrixia crinita* | E Esterhuysen 29942 | Sneeugat, Tulbagh valley | BOL | - | X | - | - |
| *Athrixia elata (a)* | SP Bester 6919 | Waterkloof ridge, Pretoria | PRE | X | X | X | - |
| *Athrixia elata (b)* | NG Bergh 2203 | Dullstroom | NBG | X | X | X | X |
| *Athrixia fontana (a)* | M Koekemoer 3554 | Sehlabathebe N. P. Lesotho | PRE | X | X | X | - |
| *Athrixia fontana (b)* | NG Bergh 1508 | Sani Pass Drakensberg | NBG | X | - | X | - |
| *Athrixia fontinalis* | Grosvenor 191 | Mozambique | K | - | X | - | - |
| *Athrixia gerardii* | NG Bergh 1523 | Utrecht | NBG | X | X | - | - |
| *Athrixia heterophylla (a)* | D Euston-Brown sn | Kouga mountains | BOL | X | X | - | - |
| *Athrixia heterophylla (b)* | P Goldblatt 11634 | Groenland mountains Caledon | NBG | - | X | - | X |
| *Athrixia heterophylla (c)* | P Goldblatt & LJ Porter 12493 | Joubertina | NBG | - | X | - | X |
| *Athrixia phylicoides (a)* | Romo et al. 14395 | Rhodes | PRE | X | X | - | X |
| *Athrixia phylicoides (b)* | NG Bergh 2180 | Cathedral Peak Drakensberg | NBG | X | X | - | - |
| *Athrixia cf.rosmarinifolia (a)* | CK Willis & JM Luhanga | Nyika National Park | PRE | - | X | X | - |
| *Athrixia rosmarinifolia (b)* | WJJO De Wilde 10436 | Ethiopia near Geddo | BOL | X | X | - | - |
| *Athrixia subsimplex* | Pócs 6752/C | Malawi | K | - | X | - | - |
| *Arrowsmithia styphelioides (a)* | OM Hilliard & BL Burtt 13266 | Katberg Pass | PRE | X | X | - | - |
| *Arrowsmithia styphelioides (b)* | NG Bergh 2129 | Katberg Pass | NBG | X | X | X | - |
| *Arrowsmithia styphelioides (c)* | NG Bergh 2188 | Stutterheim | NBG | X | X | X | - |
| *Comborhiza longipes* | E Esterhuysen 30022 | Sneeugat, Tulbagh valley | BOL | - | X | - | - |
| *Comborhiza virgata (a)* | NG Bergh 2174 | Injisuthi Drakensberg | NBG | X | X | X | - |
| *Comborhiza virgata (b)* | NG Bergh 2179 | Injisuthi Drakensberg | NBG | X | X | X | X |
| *Comborhiza virgata (c)* | NG Bergh 2195 | Injisuthi Drakensberg | NBG | X | X | X | X |
| *Leysera gnaphalodes (a)* | Romo et al.14546 | Worcester, Over Hex | PRE | X | X | - | X |
| *Leysera gnaphalodes (b)* | M Koekemoer 3313 | Namaqua National Park | PRE | - | X | - | X |
| *Leysera leyseroides* | Lambinon 22077 | Morocco | PRE | - | X | X | X |
| *Leysera tenella* | NG Bergh 1554 | Kamieskroon | NBG | X | X | - | - |
| *Macowania abyssinica (a)* | Polunin 11650 | Ethiopia | K | - | X | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Macowania abysinnica (b)* | Friis et al. 12210 | Ethiopia | K | - | X | X | - |
| *Macowania conferta (a)* | NG Bergh 2245 | Ngeli Mountain, Kokstad | K | X | X | X | - |
| *Macowania conferta (b)* | NG Bergh 2246 | Ngeli Mountain, Kokstad | NBG | X | X | X | - |
| *Macowania corymbosa (a)* | J Bentley 002 | Cathedral Peak Drakensberg | NBG | X | X | X | - |
| *Macowania corymbosa (b)* | ARA Noel 1672 | Sinyazi | GRA | X | X | - | - |
| *Macowania corymbosa (c)* | NG Bergh 2177 | Injisuthi Drakensberg | NBG | X | X | X | - |
| *Macowania deflexa (a)* | NG Bergh 2173 | Injisuthi Drakensberg | NBG | X | X | X | - |
| *Macowania deflexa (b)* | NG Bergh 2178 | Injisuthi Drakensberg | NBG | X | X | X | - |
| *Macowania ericifolia* | Miller 3313 | Yemen | K | - | X | - | - |
| *Macowania glandulosa (a)* | OM Hilliard & BL Burtt 17984 | Sani Pass Drakensberg | NU | X | X | - | - |
| *Macowania glandulosa (b)* | NG Bergh 2181 | Sani Pass Drakensberg | NBG | X | X | X | - |
| *Macowania hamata (a)* | JP Roux 1826 | Sani Pass Drakensberg | NBG | X | X | X | - |
| *Macowania hamata (b)* | NG Bergh 2166 | Sani Pass Drakensberg | NBG | X | X | X | - |
| *Macowania hamata (c)* | CJ Ward 10145 | Sani Pass Drakensberg | PRE | X | X | X | - |
| *Macowania pinifolia (a)* | J Bentley 003 | Royal Natal Drakensberg | NBG | X | X | X | X |
| *Macowania pinifolia (b)* | J Bentley 004 | Royal Natal Drakensberg | NBG | X | X | - | - |
| *Macowania pinifolia (c)* | MP Robertson 74 | Sani Pass Drakensberg | PRE | X | X | X | - |
| *Macowania pinifolia (d)* | TD Abbot 7875 | Garden Castle Drakensberg | PRE | X | X | X | - |
| *Macowania pulvinaris (a)* | JE Victor 1569 | Barkly East | PRE | X | X | X | - |
| *Macowania pulvinaris (b)* | M Koekemoer 1581 | Rhodes | PRE | X | X | X | - |
| *Macowania pulvinaris (c)* | NG Bergh 2140 | Naudesnek, Maclear | NBG | X | X | X | - |
| *Macowania revoluta (a)* | J Bentley 001 | Pirie Forest Reserve | NBG | X | X | X | X |
| *Macowania revoluta (b)* | J Bentley 005 | Pirie Forest Reserve | NBG | X | X | X | - |
| *Macowania sororis (a)* | TR Green 1237 | Sani Pass Drakensberg | NU | X | X | X | - |
| *Macowania sororis (b)* | FK Hoener 1714 | Sehlabathebe N. P. Lesotho | NU | X | X | - | - |
| *Macowania sororis (c)* | NG Bergh 2161 | Mount Currie, Kokstad | NBG | X | X | X | - |
| *Macowania tenuifolia (a)* | M Koekemoer 2079 | Mount Sheba | PRE | - | X | X | - |
| *Macowania tenuifolia (b)* | M Koekemoer 2100 | Mashishing | PRE | X | X | X | - |
| *Macowania tenuifolia (c)* | NG Bergh 2211 | Mount Sheba, Pilgrim's Rest | NBG | X | X | X | X |
| *Nestlera biennis* | NG Bergh 1819 | Elandsbaai | NBG | X | X | X | X |
| *Oedera capensis (a)* | M Koekemoer 3731 | Gansbaai | PRE | X | X | X | - |
| *Oedera capensis (b)* | NG Bergh 1771 | Bontebok National Park | NBG | X | X | - | - |
| *Oedera capensis (c)* | M Koekemoer 4051 | - | PRE | X | X | X | X |
| *Oedera conferta* | NG Bergh 2103 | Kamiesberg Peak | NBG | X | X | X | - |
| *Oedera epaleacea (a)* | JBP Beyers 272 | Ceres | PRE | X | X | X | X |
| *Oedera epaleacea (b)* | NG Bergh 1748 | Swartruggens | NBG | X | X | X | X |
| *Oedera genistifolia (a)* | J Bentley 264 | Hessequa Reserve | BOL | X | X | X | X |
| *Oedera genistifolia (b)* | J Bentley 295 | Gifberg | BOL | X | X | X | - |
| *Oedera genistifolia (c)* | M Koekemoer 2336 | Grahamstown | PRE | X | X | X | - |
| *Oedera genistifolia (d)* | NG Bergh 1572 | Grahamstown | NBG | X | X | X | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Oedera hirta* | HC Taylor 9732 | Groot Winterhoek Peak | PRE | X | X | X | X |
| *Oedera imbricata (a)* | M Koekemoer 1436 | Rooiberg Pass Calitzdorp | PRE | X | X | - | - |
| *Oedera imbricata (b)* | NG Bergh 1754 | De Hoop Nature Reserve | NBG | - | X | X | X |
| *Oedera multipunctata (a)* | CL Bellamy 17 | Botterkloof Pass | PRE | X | X | - | - |
| *Oedera multipunctata (b)* | NG Bergh 1808 | Northern Cederberg | NBG | X | X | - | - |
| *Oedera nordenstamii* | NG Bergh 2120 | Richtersveld National park | NBG | X | X | X | X |
| *Oedera resinifera (a)* | MB Bayer 6102 | Montagu. Grootvlakte Farm. | NBG | X | X | X | - |
| *Oedera resinifera (b)* | NG Bergh 2044 | Garcia's Pass, Langeberg | NBG | X | X | X | X |
| *Oedera sedifolia (a)* | J Bentley 015 | Swartberg Pass | NBG | X | X | - | - |
| *Oedera sedifolia (b)* | M Koekemoer 3837 | Langkloof Kamiesberg | PRE | X | X | - | X |
| *Oedera sedifolia (c)* | NG Bergh 1745 | Swartruggens | NBG | X | X | X | - |
| *Oedera sp.* | NA Helme 4887 | Kwadousberg, Witvlakte | NBG | X | X | - | X |
| *Oedera steyniae* | NG Bergh 1762 | Vermaaklikheid | NBG | X | X | X | - |
| *Oedera silicicola* | NG Bergh 2088 | Knersvlakte, Vredendal | NBG | X | X | - | - |
| *Oedera squarrosa (a)* | J Bentley 193 | Villiersdorp | BOL | X | X | X | X |
| *Oedera squarrosa (b)* | M Koekemoer 3634 | Between Biedouw/Pakhuis Pass | PRE | X | X | X | X |
| *Oedera squarrosa (c)* | J Bentley 282 | Gifberg | BOL | X | X | X | X |
| *Oedera squarrosa (d)* | NG Bergh 1065 | Karoopoort | NBG | X | X | X | X |
| *Oedera uniflora (a)* | M Koekemoer 4050 | - | PRE | X | X | X | X |
| *Oedera uniflora (b)* | JJ Meyer 1787 | Bredasdorp | PRE | X | X | X | - |
| *Oedera uniflora (c)* | NG Bergh 1597 | Napier | NBG | X | X | X | - |
| *Oedera viscosa (a)* | NA Helme 3151 | Malmesbury | NBG | X | X | - | X |
| *Oedera viscosa (b)* | NG Bergh 1813 | Gifberg Pass, Maskam | NBG | X | X | X | - |
| *Oreoleysera montana* | NA Helme 2863 | Hex River mountains | NBG | - | X | - | - |
| *Pentatrichia alata (a)* | NG Bergh 2209 | Pilgrims Rest | NBG | X | X | X | - |
| *Pentatrichia alata (b)* | H Burn 9016 | Pilgrims Rest | LYD | X | X | - | - |
| *Pentatrichia avasmontana (a)* | E Klaassen & Hochobes 2146 | Aredareigas N.R. Khomas | WIND | X | X | X | - |
| *Pentatrichia avasmontana (b)* | E Klaassen & Hochobes 2147 | Aredareigas N.R. Khomas | WIND | X | X | X | - |
| *Pentatrichia avasmontana (c)* | Seydel sn | Windhoek bergland | B | X | X | - | - |
| *Pentatrichia integra* | NG Bergh 2059 | Seweweekspoort, Groot Swartberg | NBG | - | X | X | - |
| *Pentatrichia kuntzei (a)* | NG Bergh 2075 | Cogmanskloof, Montagu | NBG | X | X | X | - |
| *Pentatrichia kuntzei (b)* | RJ Bayer& CF Puttock sn | Kogmanskloof Pass | WIND | X | X | X | - |
| *Pentatrichia petrosa (a)* | E Klaassen & Hochobes 1745 | Waterberg Plateau Park | WIND | X | X | X | - |
| *Pentatrichia petrosa (b)* | E Klaassen & Hochobes 1747 | Waterberg Plateau Park | WIND | X | - | X | - |
| *Pentatrichia petrosa (c)* | E Klaassen & Hochobes 2143 | Brandberg, Erongo | WIND | X | X | X | - |
| *Pentatrichia petrosa (d)* | Giess 3949 | Klein Windhoek | WIND | X | X | X | - |
| *Pentatrichia rehmii (a)* | E Klaassen & Hochobes 1751 | Guchab mountain | WIND | X | X | X | - |
| *Pentatrichia rehmii (b)* | E Klaassen & Hochobes 1752 | Guchab mountain | WIND | X | X | X | - |
| *Pentatrichia rehmii (c)* | E Klaassen & Hochobes 2137 | Guchab mountain | WIND | - | X | X | - |
| *Phagnalon ballii subsp. embergeri* | Gómiz 5650 | High central Atlas, Morocco | * | X | X | X | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Phagnalon barbeyanum* | Collenette 6561 | Saudi Arabia, Harrat al Harrah | E | X | X | X | - |
| *Phagnalon bicolor* | Gómiz sn | Marrakech, Morocco | * | X | X | X | - |
| *Phagnalon graecum* | Aldasoro 3308 | Monte San Angelo, Italy | MA | X | X | X | - |
| *Phagnalon latifolium* | Gómiz sn | Maire, Morocco | * | X | X | X | - |
| *Phagnalon platyphyllus* | Sáez 6188 | Morocco | ** | X | X | X | - |
| *Phagnalon pygmaeum* | Saéz sn | Greuter, Greece | ** | X | X | X | - |
| *Phagnalon saxatilis* | Montes-Moreno et al. | Collserola, Spain | BCN | X | X | X | X |
| *Phagnalon sordidum* | Montes-Moreno et al. | Monteserat, Spain | BCN | X | X | X | X |
| *Phagnalon stenolepsis* | Miller 3155 | Yemen | E | X | X | X | - |
| *Phagnalon umbelliforme* | - | El Hierro, Spain | *** | X | X | X | - |
| *Relhania acerosa (a)* | M Koekemoer 2067 | Naudesnek | PRE | X | X | X | X |
| *Relhania acerosa (b)* | NG Bergh 2137 | Naudesnek, Maclear | NBG | X | X | - | X |
| *Relhania acerosa (c)* | PC Zietsman 3536 | Qwa Qwa National Park | PRE | X | X | - | X |
| *Relhania calycina (a)* | J Bentley 10 | Swartberg Pass | NBG | X | X | X | X |
| *Relhania calycina (b)* | M Koekemoer 3471 | Swartberg Pass | PRE | X | X | X | - |
| *Relhania corymbosa* | NG Bergh 1795 | Witteberg, Laingsburg | NBG | - | X | X | X |
| *Relhania dieterlenii (a)* | M.S.Mothogoane 127 | Rhodes | PRE | X | X | - | X |
| *Relhania dieterlenii (b)* | NG Bergh 2148 | Rhodes | NBG | X | X | X | - |
| *Relhania garnotii (a)* | P Goldblatt 5617 | Bredasdorp | PRE | X | X | X | - |
| *Relhania garnotii (b)* | NG Bergh 1755 | Potberg | NBG | X | X | X | X |
| *Relhania fruticosa (a)* | J Bentley 471 | Romansrivier | NBG | X | X | - | X |
| *Relhania fruticosa (b)* | J Manning 3365 | Elandsberg | NBG | - | X | - | X |
| *Relhania pungens (a)* | C.L.Bredenkamp 3353 | Mpofu Game Reserve | PRE | X | X | X | X |
| *Relhania pungens (b)* | M Koekemoer 3430 | Garcias Pass | PRE | X | X | - | - |
| *Relhania pungens (c)* | NG Bergh 1772 | Bontebok National Park | NBG | X | X | X | X |
| *Relhania relhanioides (a)* | P Goldblatt 4176 | Laingsburg | PRE | X | X | X | X |
| *Relhania relhanioides (b)* | NG Bergh 2067 | Touwsriver | NBG | X | X | - | X |
| *Relhania rotundifolia (a)* | NA Helme 5585 | Hopefield | NBG | X | X | - | X |
| *Relhania rotundifolia (b)* | T Oliver sn | Riverlands | **** | X | X | - | X |
| *Relhania spathulifolia* | NG Bergh 1777 | Elim | NBG | X | X | X | X |
| *Relhania speciosa (a)* | C.L.Bredenkamp 841 | Touwsberg | PRE | X | X | X | X |
| *Relhania speciosa (b)* | J Bentley 11 | Swartberg Pass | NBG | X | X | - | - |
| *Relhania speciosa (c)* | NG Bergh 1797 | Witteberg, Laingsburg | PRE | X | - | X | X |
| *Relhania tricephala (a)* | Koekemoer 489 | Waboomsberge | PRE | X | X | - | - |
| *Relhania tricephala (b)* | NG Bergh 1790 | Ceres Valley | NBG | X | X | X | X |
| *Rhynchopsidium pumilim* | H.M.Steyn 1328 | Namaqua National Park | PRE | - | X | X | X |
| *Rhynchopsidium sessiliflorum (a)* | M Koekemoer 2404 | Blinkberg Pass | PRE | X | X | X | X |
| *Rhynchopsidium sessiliflorum (b)* | NG Bergh 2062 | Touwsriver | NBG | - | X | X | X |
| *Rosenia glandulosa* | NG Bergh 1729 | Calvinia | NBG | X | X | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Rosenia humilis (a)* | EP Nienaber 427 | Near Middelburg | PRE | X | - | X | X |
| *Rosenia humilis (b)* | M Koekemoer 2865 | Victoria West and Britstown | PRE | X | X | X | - |
| *Rosenia oppositifolia (a)* | M Koekemoer 2771 | Salpeterkop, Sutherland | PRE | X | X | - | - |
| *Rosenia oppositifolia (b)* | NG Bergh 1705 | Roggeveld escarpment | NBG | X | X | - | - |
| *Rosenia spinescens (a)* | B sn | - | PRE | X | X | X | - |
| *Rosenia spinescens (b)* | NG Bergh 1740 | Roggeveld escarpment | NBG | X | X | X | X |
| **Outgroup and non-Relhanioid Gnaphalieae species** | | | | | | | |
| *Amphiglossa corrudifolia* | M Koekemoer 1291 | Prince Albert | PRE | X | X | X | X |
| *Calendula officinalis* | CHR 542251 | - | CHR | X | X | - | X |
| *Cotula coronopifolia* | CHR 518831 | - | CHR | X | X | - | X |
| *Dimorphotheca cuneata* | NG Bergh1722B | Karoopoort | NBG | - | X | X | X |
| *Dolichothrix ericoides* | NG Bergh 180 | Cederberg | NBG | X | X | - | - |
| *Felicia filifolia* | Lowrey 2147 | - | - | X | - | - | X |
| *Gamochaeta subfalcata* | Galbany et al | Spain | BCN | X | X | - | - |
| *Garuleum bipinnatum* | Goldblatt P. & Porter L. 12557 | - | NBG | X | X | X | X |
| *Ifloga molluginoides* | NG Bergh 1680 | Steinkopf | NBG | X | - | - | X |
| *Ifloga spicata* | Lambinon 17590 | Morocco, Errachidia | PRE | X | X | X | X |
| *Lasiopogon glomerulatus* | NG Bergh 1645 | Knersvlakte, Vanrhynsdorp | NBG | X | - | - | X |
| *Osmitopsis cf asteriscoides.* | NG Bergh 2215 | Ex hort Kirstenbosch | NBG | - | X | X | X |
| *Philyrophyllum brandbergense (a)* | E Klaassen 2141 | Nambia | WIND | X | X | X | - |
| *Philyrophyllum brandbergense (b)* | E Klaassen 2142 | Nambia | WIND | X | X | X | - |
| *Philyrophyllum brandbergense (c)* | E Klaassen 2144 | Nambia | WIND | X | X | X | - |
| *Philyrophyllum schinzii (a)* | E Klaassen 1371 | Nambia | WIND | X | X | X | - |
| *Philyrophyllum schinzii (b)* | E Klaassen 2256 | Nambia | WIND | X | X | X | X |
| *Philyrophyllum schinzii (c)* | E Klaassen 2260 | Nambia | WIND | X | X | X | X |
| *Printzia auriculata* | NG Bergh 2199 | Mlambonja Valley, Drakensberg | NBG | - | X | X | X |
| *Stoebe cinerea* | NG Bergh 1210 | Houwhoek, W. Cape | NBG | X | - | - | - |
| *Syncarpha canescens* | S Haiden 021 | Near Ceres | NBG | X | X | X | X |
| *Senecio vulgaris* | P Pelser 188 | Austria | - | - | X | - | X |

* Gómiz Pers. Herb.
** Saéz Pers. Herb.
*** Ex hort. Bot. Viera & Clavijo
**** T. Oliver CREW fieldtrip

# Chapter Three: Estimating pathways of dispersal: the palaeoenvironmental migration of Afrotemperate and arid relhanioid lineages

## Introduction

The temperate flora of the Greater Cape Floristic Region (GCFR: Born et al. 2007; Jürgens 1997), represented mostly in the summer-arid and semi-desert climates of South Africa, is renowned for its remarkable species-richness and endemism (Cowling et al. 1992; Goldblatt 1978; Goldblatt & Manning 2002). This flora has close ties to other regions, with GCFR "elements" (or, clades) represented in the adjacent arid Nama Karoo and Namib Desert regions (Cowling et al. 1989; Jürgens 1997), the Afrotemperate regions along the eastern escarpment of Africa (Linder 1990; White 1978, 1984), and the arid and mediterranean zones of the circum-Mediterranean region. The distributions of plant lineages across these regions are often discontinuous, however, associating with islands of suitable habitat which are separated by intervening areas of unsuitable habitat. One suite of lineages occupy arid habitats, and the other, Afrotemperate habitats. Lineages may be disjunct along the mesic Afrotemperate mountain ranges of East Africa and the Drakensberg, or between these and Afrotemperate forest pockets in the GCFR and alpine-type habitats of the circum-Mediterranean. Examples of these range disjunctions occur in *Disa, Pentaschistis* (Galley et al. 2007), *Euryops* (Devos et al. 2010) and *Scabiosa* (Carlson et al. 2012). Disjunctions may also occur between the circum-Mediterranean and arid regions of southwestern Africa. Examples include *Zygophylloideae* (Bellstedt et al. 2012), *Rhinusa* and *Gymnetron* (Hernández-Vera et al. 2013), *Androcymbium* (del Hoyo et al. 2009) and *Senecio* (Coleman et al. 2003).

Three historical scenarios might explain these disjunct distribution patterns. Populations may have been historically connected between these regions, the present-day discontinuities being the result of historical vicariance. Alternatively, these discontinuities might be explained by long-distance

dispersal over areas of unsuitable habitat. Another possible scenario is repeated short-distance dispersal aided by the existence of "stepping-stones", or, isolated areas of suitable habitat, often mountains, which facilitate step-wise dispersal. Whether these disjunctions are explained by the fragmentation of once-widespread ranges, long-distance dispersal or "stepping-stone" dispersal, many of the lineages studied to date appear to have originated in southern Africa with northwards migration into the eastern Afrotemperate, arid or circum-Mediterranean regions (Bellstedt et al. 2012; Calviño et al. 2006; Caujapé-Castells et al. 2001; Coleman et al. 2003; del Hoyo et al. 2009; Galley et al. 2007; Hernández-Vera et al. 2013). In some lineages, however, range expansion appears to have proceeded from north to south (Carlson et al. 2012; Gehrke & Linder 2009; McGuire & Kron 2005).

Given the tendency for lineages to track their ecological niches than adapt to new ones, a theory called phylogenetic niche conservatism (Kozak & Wiens 2006; Wiens 2004; Wiens et al. 2010), the distribution of suitable habitat may be critical in dictating the ultimate distributions of individual lineages. For instance, Crisp et al. (2009) reconstructed the ancestral habitats of several lineages of extratropical flora and found that biome stasis at speciation far outweighed biome shifts, at a ratio of 25:1. As has been demonstrated previously (Schrire et al. 2005; Thiv et al. 2011), widely-distributed clades should occupy analogous habitats in distant areas. From this line of evidence it might be expected that, in the majority of cases, arid-adapted and Afrotemperate-adapted lineages are likely to trace distinct routes within the confines of their environmental niche. This has been found to be the case in the migration of several lineages (e.g. Lavin et al. 2004; Galley et al. 2007; Schrire et al. 2005; Thiv et al. 2011).

Based largely on insights from biogeographic studies and bolstered by paleoclimatic and geological evidence, the existence of an "arid track", a dispersal route between the arid and mediterranean-

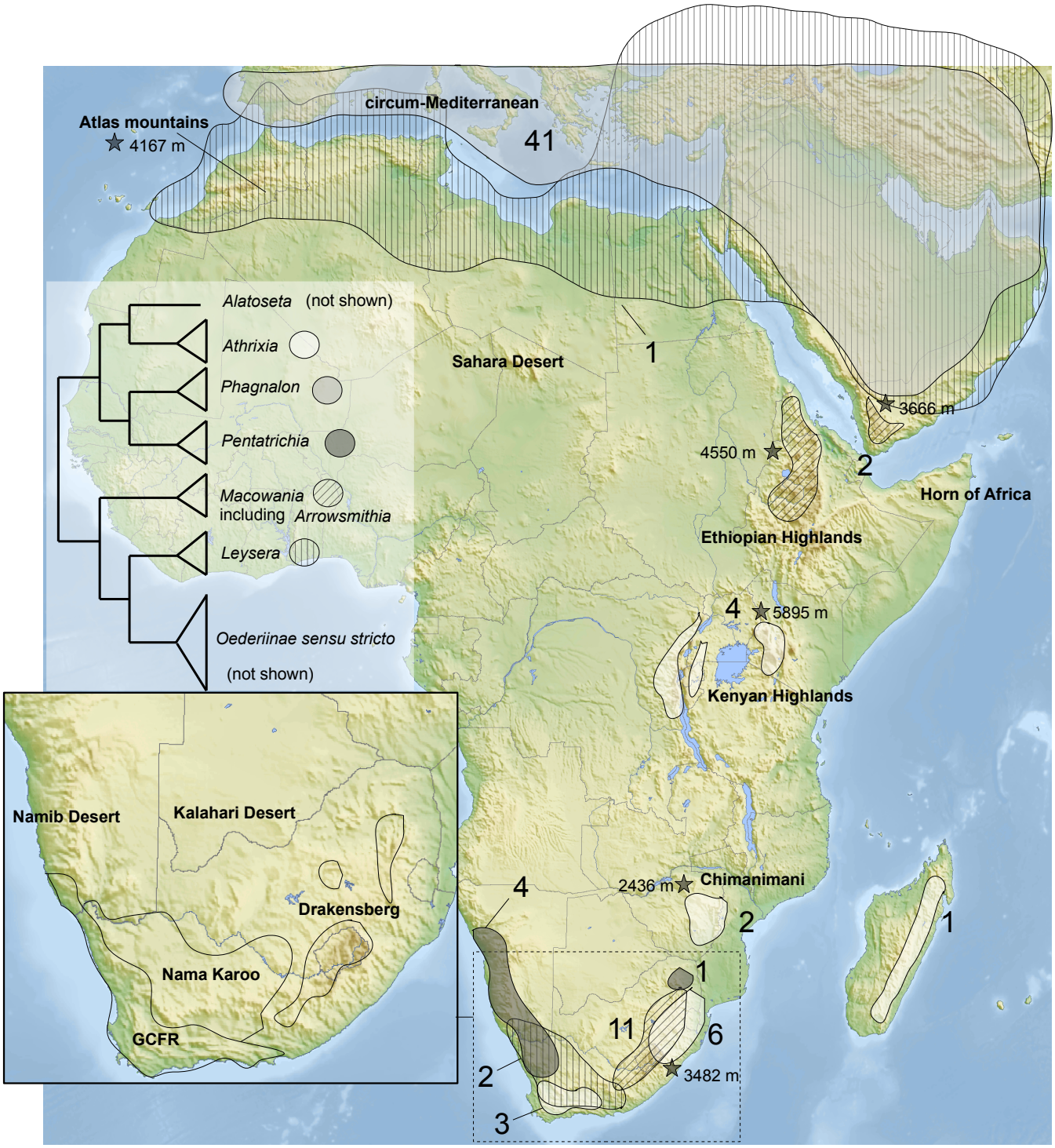



**Fig. 3.1. Topographic map indicating the major range disjunctions exhibited by members of the two subclades, Athrixiinae and Oederiinae.**

The numbers next to the polygons indicate the number of species therein (and in some instances, the same species might occur across two disjunctions). The stars with associated value (m) indicate the highest elevation of the montane regions.

climate areas of southwestern Africa and the circum-Mediterranean, has been proposed (Balinsky 1962; Jürgens 1997). While this pattern has commonly been explained in terms of long-distance dispersal (Coleman et al. 2003; Raven 1973; Thiv et al. 2011; Thorne 1972), most work invokes a putative physical corridor of arid habitat, which may have been continuous or semi-contiguous (i.e. via stepping-stones), between these regions (e.g. Bellstedt et al. 2012; Calviño et al. 2006; Caujapé-Castells et al. 2001; del Hoyo et al. 2009; Hernández-Vera et al. 2013). The arid corridor is believed to have existed since the end of the Miocene and best developed during the glacial cycles of the Pleistocene (Goldblatt 1978; van Zinderen Bakker 1975; Verdcourt 1969). It is probable that the corridor existed periodically, opening and closing along with fluctuating climate and intermittent periods of aridity. This is substantiated by molecular dating studies, revealing periodic migrations of disjunct plant and animal lineages, such as *Zygophylloideae* (Bellstedt et al. 2012), *Androcymbium* (Caujapé-Castells et al. 2001; del Hoyo et al. 2009), *Senecio* (Coleman et al. 2003), *Thamnosma* (Thiv et al. 2011), *Rhinusa* and *Gymetron* (Hernández-Vera et al. 2013), during the Miocene, Pliocene and Pleistocene. The timing of these migrations broadly correspond to estimates of historically arid conditions on the African continent since the Early Miocene (Zachos et al. 2001; Zachos et al. 2008) and in the circum-Mediterranean since the Middle to Late Miocene (Ivanov et al. 2002; Fortelius et al. 2006; Van Dam 2006). The progressive hyper-aridification of southwestern Africa is thought to have begun in the Late Miocene (DuPont et al. 2011; Diekmann et al. 2003; Linder et al. 2003; Siesser 1980; Zachos et al. 2001), with arid conditions being recorded in sub-tropical Africa (deMenocal 2004), in Namibia (DuPont et al. 2005; DuPont 2006) and in the Horn of Africa (Trauth et al. 2009) at several intervals during the Pleistocene.

A mesic Afrotemperate track from the GCFR along the eastern escarpment mountain ranges as far as Yemen may have functioned as a corridor for the movement of currently-disjunct Afrotemperate elements. While some studies infer northward migrations from the Cape into the Afrotemperate

regions (Devos et al. 2010; Galley et al. 2007; Sanmartín et al. 2010), there is also evidence for southwards colonisation by European temperate taxa (Carlson et al. 2012; Gehrke & Linder 2009; McGuire & Kron 2005). It remains unclear whether the Afrotemperate track represents a long-distance dispersal route (e.g. Carlson et al. 2012), a relict of a once-continuous corridor fragmented by changing climate (Adamson 1958; Chrowics 2005; King 1978; Wild 1968) or, the most widely-accepted theory, a series of "stepping-stones" supporting the transport of taxa between Afrotemperate peaks, or sky-islands. The Drakensberg in particular has been cited as an important connection (Carlson et al. 2012; Galley et al. 2007; Holland 1978; Linder 1990, 1994; Sanmartín et al. 2010; Weimarck 1941). An inference of "stepping-stone" migration along the Afrotemperate track would require that the appearance of high-altitude habitats of the East African Highlands predate the dispersal of Afrotemperate lineages into these regions. The product of rift-flank uplift (flexing of the Earth's surface associated with rifting), the East African Highlands have existed since the Middle Miocene, with some peaks rifting as late as the Pleistocene (Chorowics 2005; Flannery & Rosendahl 1990; Wolfenden et al. 2004). The elevation of the Drakensberg range has been attributed to two tectonic events during the Early Miocene and Early Pliocene (Partridge & Maud 1987). Dated phylogenetic studies of typical Afrotemperate-disjunct lineages are few, but one study of four Afrotemperate floral clades (Galley et al. 2007) overwhelmingly supports a "stepping-stone" hypothesis with several northwards migrations from the GCFR into East Africa, primarily via the Drakensberg. Here, numerous independent dispersals occur at times ranging from the Miocene to Early Pleistocene. A Pleistocene dated migration into East Africa in *Euryops* from southern African mountain ranges augments support for this (Devos et al. 2010).

The *Relhania* clade is well-represented in the Afrotemperate and arid regions of Africa and the circum-Mediterranean. Several range disjunctions along putative arid and Afrotemperate tracks are also evident, presenting opportunities to test the migration scenarios underlying these disjunctions.

Within Athrixiinae, *Athrixia* is distributed from the GCFR to the highlands of East Africa and Madagascar, attaining its greatest diversity along the eastern Great Escarpment (Drakensberg) of South Africa (11 species: Fig. 3.1). In Oederinae, *Macowania* (including *Arrowsmithia*) has its greatest diversity in the Drakensberg, hosting 11 species out of 13, the remaining two being disjunct in the highlands of Ethiopia, Eritrea Djibouti and Yemen. The *Relhania* clade also offers opportunities to explore arid track affinities. The Namibian-centred *Pentatrichia* clade is sister to a clade comprising *Phagnalon* (41 spp.), the latter being confined to the Northern Hemisphere and occurring throughout the circum-Mediterranean as far east as Pakistan (Fig. 3.1). The small genus *Leysera* occurs in the GCFR and surrounding arid areas of southern Africa with one species disjunct in the Northern Hemisphere and exhibiting a circum-Mediterranean distribution, from the Atlas Mountains to Pakistan (Fig. 3.1).

Modern Bayesian methods of phylogenetic inference provide a powerful means of estimating species relationships and dating the successive speciation events underlying present-day species diversity by means of a molecular clock. The addition of likelihood-based biogeographic analysis as implemented in Lagrange (Ree et al. 2005; Ree & Smith 2008) provides a framework for testing alternative hypotheses relating to the origin and directionality of dispersal, using a dispersal-extinction-cladogenesis (DEC) model of lineage evolution. The DEC model allows for dispersal events between certain areas to be disallowed, corresponding to particular dispersal scenarios. The likelihoods of the reconstructions generated under different dispersal scenarios can be compared, enabling an assessment of which scenario best explains the observed present day distributions. Using these tools, the following hypotheses will be tested:

1) The *Relhania* clade originated in the GCFR, its modern pan-African distribution being the result of northward migration.

2) Northward dispersal from the GCFR proceeded via stepping-stones, these being distinct for the arid and Afrotemperate tracks. In the case of the arid track, dispersal may have proceeded via the Namaqualand-Namib domain, whereas in the case of the Afrotemperate track, dispersal commenced via the greater Drakensberg region.
3) Alternatively, northward dispersal proceeded via continuous arid and Afrotemperate corridors whose existence may have been temporary, coinciding with periods of aridity and continental elevation, respectively.
4) Migration via the arid and mesic Afrotemperate tracks has proceeded with little change in the moisture niches of the lineages involved.

# Materials and Methods

### Estimation of divergence dates

The same taxon set was used as in Chapter Two. Divergence dates were estimated using an uncorrelated lognormal (UCLN) clock to model molecular rate variation among branches (Drummond et al. 2006) as implemented in BEAST V. 1.7.4 (Drummond & Rambaut 2007). In the absence of direct fossil calibration, the analysis was calibrated using node age estimates (and associated errors) obtained from a higher-level fossil-calibrated molecular clock analysis of the family Asteraceae by Hoffmann (2012). The uncertainty associated with secondary calibrations can be directly incorporated in BEAST, through the specification of appropriate priors. For instance, a normal prior distribution, the rationale being that uncertainty is likely to be evenly distributed on either side of the age used (Forest 2009; Shaul & Graur 2002). Hoffmann (2012) calibrated a phylogeny of the family Asteraceae using (i) a secondary calibration point for the root height, obtained from the higher-level phylogeny of Bremer et al. (2004), and (ii) a selection of five Asteraceae fossils that have been described and verified in the literature (i.e. those fossils which were found to be erroneous and which were discredited by later studies were not included).

Included fossils were: (i) an *Artemisia*-type pollen representing the stem node of Anthemideae (Graham 1996); (ii) an *Ambrosia*-type pollen for the stem node of Heliantheae *sensu lato* (Graham 1996); (iii) a *Dicoma*-type pollen attached to the stem node of Dicomeae (Scott et al. 2006); (iv) *Mutisiapollis patersonii* pollen representing the stem node of Mutisieae (Martinez-Millan 2010; Barreda et al. 2010); and (v) a fossilised capitulum representing the split of Barnadesioideae – Mutisioideae *sensu lato* and Carduoideae (Barreda et al. 2010b). The BEAST.xml file used in this study was configured using BEAUTi V. 1.7.4 (Drummond & Rambaut 2007), implementing a mixed model in which substitution model structure and parameters were estimated separately for each data partition. The dataset was partitioned as per the MrBayes analysis (consisting of the individual gene regions ITS, ETS, *trnL-F* and *trnT-L*) in Chapter Two, and analysed using the same substitution models, implementing a random starting tree. A Yule process (a constant rate of speciation per lineage) was used to generate the tree prior. The following prior age distributions obtained from Hoffmann (2012) were used for the analysis, constraining the priors to follow a normal distribution: the mean of the root node was set at 31.6 Ma with a 95% prior confidence interval (CI) of 27.9 – 35.4 Ma, spanning the Oligocene to Late Eocene, and corresponds to the split between Senecioneae and the rest of the lineages sampled. The stem age of Gnaphalieae was calibrated with a mean of 23.0 Ma (95% CI: 18.1 – 27.9: Early Miocene to Oligocene), and the third point (the split between *Gamochaeta* and *Syncarpha,* representing the Gnaphalieae crown radiation) had a mean set at 8.0 (95% CI: 1.0 – 15.2: Pleistocene–Middle Miocene). Gamma distributed priors with scale=1.0 and offset=1.0 were set on the means of the uncorrelated relaxed clocks of each partition, otherwise all other priors were kept at their default settings.

Several short, preliminary BEAST runs were initiated to examine MCMC chain performance, after which four runs of $30^7$ generations were implemented, with sampling occurring every 1,000 generations. The output files were tested for convergence using Tracer V. 1.5 (Rambaut &

Drummond 2007) to evaluate the ESS scores, parameter estimates and the likelihood traces. Based on this assessment, the first 10% of samples from each run were discarded as burn-in. The standard deviation of the uncorrelated lognormal relaxed clock (ucld.stdev), which gives an indication of the "clock-like" behaviour of the data (Drummond et al. 2006), had a value of 0.78, indicating non-clocklike evolution and justifying the use of a relaxed clock. The maximum clade credibility tree annotated with median node heights was generated using LogCombiner V. 1.7.4 (Rambaut & Drummond 2007) and TreeAnnotator V. 1.7.4 (Rambaut & Drummond 2007).

## Biogeographic scenarios

### Georeferencing and identification of geographically meaningful regions

Distributions of the South African species were determined by georeferencing herbarium specimen records from PRE, BOL and NBG, whereas for the non-South African species online herbarium specimen records were used (JSTOR Global Plants, Kew & Paris online herbaria). Georeferencing and mapping made use of ArcGIS (Desktop 10 Service Pack 2: CA: ESRI), Quantum (Q) GIS V. 1.8.0 'Lisboa' and Google Earth, incorporating all locality points including those with wide mapping confidence levels (>10,000 m). Based on the distribution maps generated, as well as a consideration of geographic disjunctions shown by this and other plant groups, five discrete regions were identified that are consistent with general distribution patterns in the *Relhania* clade and used as units for the inference of ancestral ranges. These regions were chosen to maximise area endemism and capture repeated patterns of dispersal in order to facilitate investigation of broad patterns of biogeographic history in the *Relhania* clade. In order to facilitate statistical investigation, the number of regions was restricted to five areas. These are: the GCFR (incorporating the CFR, the Namaqualand region, and the Hantam-Roggeveld-Tankwa regions); the greater Drakensberg region (including the Eastern Cape Drakensberg, the Amatole range and the Limpopo-Mpumalanga Drakensberg); the summer-rainfall arid regions of southern Africa (including the Nama Karoo and

parts of Namibia); the East African Highlands (the eastern Afrotemperate uplands, defined here as the Afrotemperate areas from the Chimanimani mountains in Zimbabwe, northwards including the highlands of Madagascar, Malawi, Kenya, Ethiopia and Eritrea and western highlands of Yemen) and circum-Mediterranean (encompassing the Mediterranean Basin, Saharo-Arabian region, Irano-Turinian region and Macronesia). Though the latter represents a large geographic area, the primary aim was to test directionality of migration and the potential importance of the Namib region as a stepping-stone between the arid regions of the north and south. Also, as only a few representatives of the northern hemispheric genera are represented, the geographic regions could not be meaningfully teased apart within the Northern Hemisphere, though an effort was nevertheless made to sample from across the range.

### Ancestral area reconstructions and migration scenarios

Biogeographic history was explored using the dispersal-extinction-cladogenesis (DEC) model as implemented in Lagrange (Ree et al. 2005; Ree & Smith 2008). Lagrange utilises branch length information to estimate the most likely combination of areas at each ancestral node on the tree, the DEC model specifying two free parameters (the instantaneous probabilities of dispersal and local extinction) which relate, respectively, to range expansion and range contraction (Ree & Smith 2008). The use of branch length information is important in the estimation of ancestral areas because the probabilities of dispersal and local extinction are expected to increase proportionally with branch length, at least where this is representative of time.

Lagrange was used to explore both the fit of alternative migration scenarios and, in the context of the best-fit model, to infer the ancestral areas at nodes. Though not all possible permutations were tested, the resulting likelihood scores indicated which parameters were consistently poorly supported. Beginning with an unconstrained model (whereby all possible dispersal scenarios are

allowed) and then restricting certain dispersal routes, different dispersal scenarios corresponding to the hypotheses set out in the introduction were assembled and tested. Based on an examination of the resulting *–ln* likelihood scores (these being a reflection of the performance of the model), dispersal pathways which performed better were noted and incorporated into the design of subsequent dispersal models, ultimately resulting in a more refined model (Ree & Smith 2008). In total, 31 dispersal models were designed and tested. For the sake of brevity, only a subset (nine) of these (representing poor, medium and high-performing scenarios) are presented in Fig. 3.2 and discussed in the text. All other scenarios tested are presented, along with their final *– ln* likelihood scores, in Appendix 1.

In scenario (i) there are no constraints, all dispersal pathways being allowed. Scenario (ii) disallows migration from the mesic Afrotemperate Drakensberg (D) and East African Highlands (EA) to the arid circum-Mediterranean (M), and vice versa. However, migration between the arid Karoo zone (K) and D/EA is allowed. Scenario (iii) excludes the possibility of D acting as a stepping-stone for migration between the GCFR (C) and EA, requiring migration between these regions to be the result of direct long-distance dispersal. Migration between EA and M is also not possible in this scenario. Scenario (iv) only permits migration between C and M to occur through EA, either via K or D. This scenario supports the Afrotemperate track hypothesis, but not the arid track hypothesis, implying that the two tracks are not distinct. Direct long-distance dispersal between C and M is again not allowed by this scenario. In scenario (v), migration along both the arid and Afrotemperate tracks is permitted, as well as between EA and M. Direct dispersal between C and M is disallowed, corresponding to an enforced arid stepping-stone model via K. Scenario (vi) disallows movement between K and M and between C and M, but allows movement between D and M, forcing both arid and mesic lineages to reach M via a D stepping-stone, again implying that the tracks are not distinct. In (vii), movement along both the arid and Afrotemperate tracks, as distinct routes, is

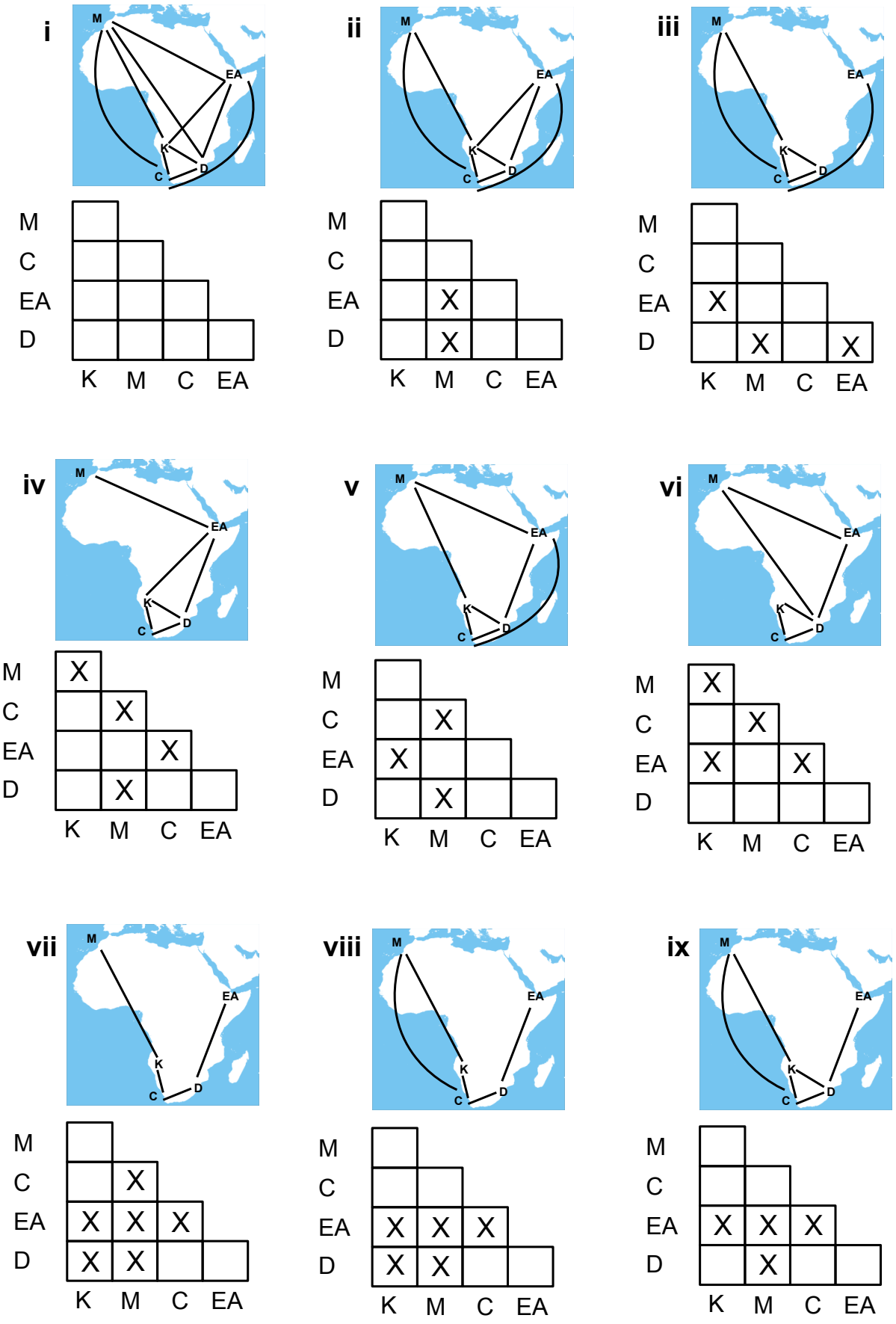



**Fig. 3.2. Maps indicating the nine migration scenarios chosen for presentation tested in the ancestral area reconstructions.**

The “X” in the tables indicates areas where dispersal was restricted, and an open box in the table indicates free movement between the areas. The letters stand for the following regions: C: GCFR, D: Drakensberg, K: Nama Karoo + Namibia, EA: East African Highlands and M: circum-Mediterranean region.

facilitated, but direct long-distance dispersal from C to EA and M is disallowed, as is movement between D and K. Scenario (viii) is similar, except that long-distance dispersal between C and M is permitted, with D enforced as a stepping-stone for the Afrotemperate taxa. Finally, scenario (ix) tests the same scenario as (viii) except that migration between D and K is allowed.

Lagrange c++ (downloaded from: http://code.google.com/p/lagrange/) was used to reconstruct ancestral areas and assess alternative migration scenarios. The Lagrange input files were compiled manually and the analyses run using the BEAST MCC tree, pruned of outgroups and multiple species accessions. The analysis was restricted to allow no more than two ancestral areas to be inferred per node. This is justified by the observation that no extant species occurs in more than two areas. Dispersal rates between areas were coded in a binary manner, either "0" (no migration allowed) or "1" (migration allowed). As such, relative rates of dispersal were not specified. The dispersal time period was set from 0 – 20 Ma, as the earliest period is required to be set as older than the given phylogeny. The different models were assessed based on their *ln*-likelihood scores, with higher scores being an indication of a more suitable model (Ree & Smith 2008).

### Habitat assignment and ancestral state reconstruction

As species might tend to track their ecological niches over time (e.g. Crisp et al. 2009; Kozak & Wiens 2006; Wiens 2004; Wiens et al. 2010), migration along separate arid or Afrotemperate tracks implies little change in the moisture niche preferences of the species involved. In other words, arid-adapted taxa would track arid corridors to disperse, while Afrotemperate taxa would probably require moister, higher altitude, corridors to achieve range expansion. In order to test this idea, species' moisture niches were quantified using mean annual precipitation (MAP) values, the values for each georeferenced specimen locality being extracted from the BIOCLIM variable 12 (http://www.worldclim.org/) in QGIS V. 1.8.0 using the "point sampling tool" plugin. A MAP

range for all species was obtained from all point localities, and summarised by the median value of all localities for each species. The median MAP values were used to infer ancestral precipitation niches on the BEAST MCC tree, trimmed of multiple species accessions, in Mesquite V. 2.75 (http://mesquiteproject.org) using squared change parsimony.

# Results

### Molecular dating analysis

### BEAST tree topology and support

The four combined BEAST log files, with the first 10% of samples discarded as burn-in, yielded 108,000,000 samples, the effective independent sample sizes associated with each parameter estimate being high (> 200). The BEAST topology was very similar to that recovered by the MrBayes and bootstrap analyses (Chapter 2) and recovered many of the same well-supported nodes (BEAST MCC tree presented in Fig. 3.3). Gnaphalieae *sensu stricto* is recovered as monophyletic (PP=0.97). There is, however, incongruence relating to the placement of *Philyrophyllum.* Identified as sister to the more distant outgroup samples in this study by MrBayes (Chapter 2: Fig. 2.5), BEAST recovered *Philyrophyllum* as sister to Gnaphalieae *sensu stricto* with PP=1.0. Calenduleae, Anthemideae and Astereae are each supported as monophyletic with PP=1.0. The *Relhania* clade is recovered as sister to the crown radiation of Gnaphalieae (PP=1.0). Within the *Relhania* clade (PP=1.0), both Athrixiinae and Oederinae are recovered with high support (PP=1.0). The parsimony bootstrap, MrBayes and BEAST analyses resolve species-level relationships within the *Relhania* clade which are largely consistent, at least with respect to well-supported nodes (PP>0.95). Two exceptions are the monophyly of *Pentatrichia,* previously unsupported in the MrBayes analyses, which is now supported with PP=0.97, and monophyly of the "Cape" clade (see Chapter Two) which is no longer supported (PP<0.95).

**Table 3.1: Crown node age estimates in millions of years including 95% HPD and PP node support values for the major clades indicated on the BEAST tree in Fig. 3.3.**

| Node | Description | Median height and 95% HPD (Ma) | PP |
|---|---|---|---|
| A | Root node | 31.6 (26.3 – 38.5) | – |
| B | Gnaphalieae *sensu stricto* | 14.1 (9.4 – 19.2) | 0.97 |
| C | Gnaphalieae | 22.1 (18.3 – 26.1) | 0.97 |
| D | *Relhania* clade | 17.7 (14.0 – 21.3) | 1.0 |
| E | Athrixiinae | 13.7 (10.2 – 17.6) | 1.0 |
| F | Oederinae | 14.3 (11.0 – 18.2) | 1.0 |
| G | *Athrixia* | 4.7 (2.7 – 7.6) | 1.0 |
| H | *Phagnalon* | 5.7 (3.6 – 8.2) | 1.0 |
| I | *Pentatrichia* | 6.9 (4.4 – 9.8) | 0.98 |
| J | *Macowania* | 11.3 (7.4 – 15.5) | 0.99 |
| K | *Leysera* | 8.0 (5.1 – 11.5) | 1.0 |
| L | Oederinae *sensu stricto* | 9.2 (6.4 – 12.9) | 0.99 |

## Divergence date estimation

The posterior estimates of the calibration nodes all closely resemble the specified prior distributions. The dates estimates for nodes of interest illustrated on Fig. 3.3 are presented in Table 3.1. The *Relhania* clade is estimated to have originated in the Early Miocene (17.7 Ma, 95% HPD: 14.0 – 21.3), with Oederinae and Athrixiinae starting to diversify from about the Middle Miocene (14 Ma).

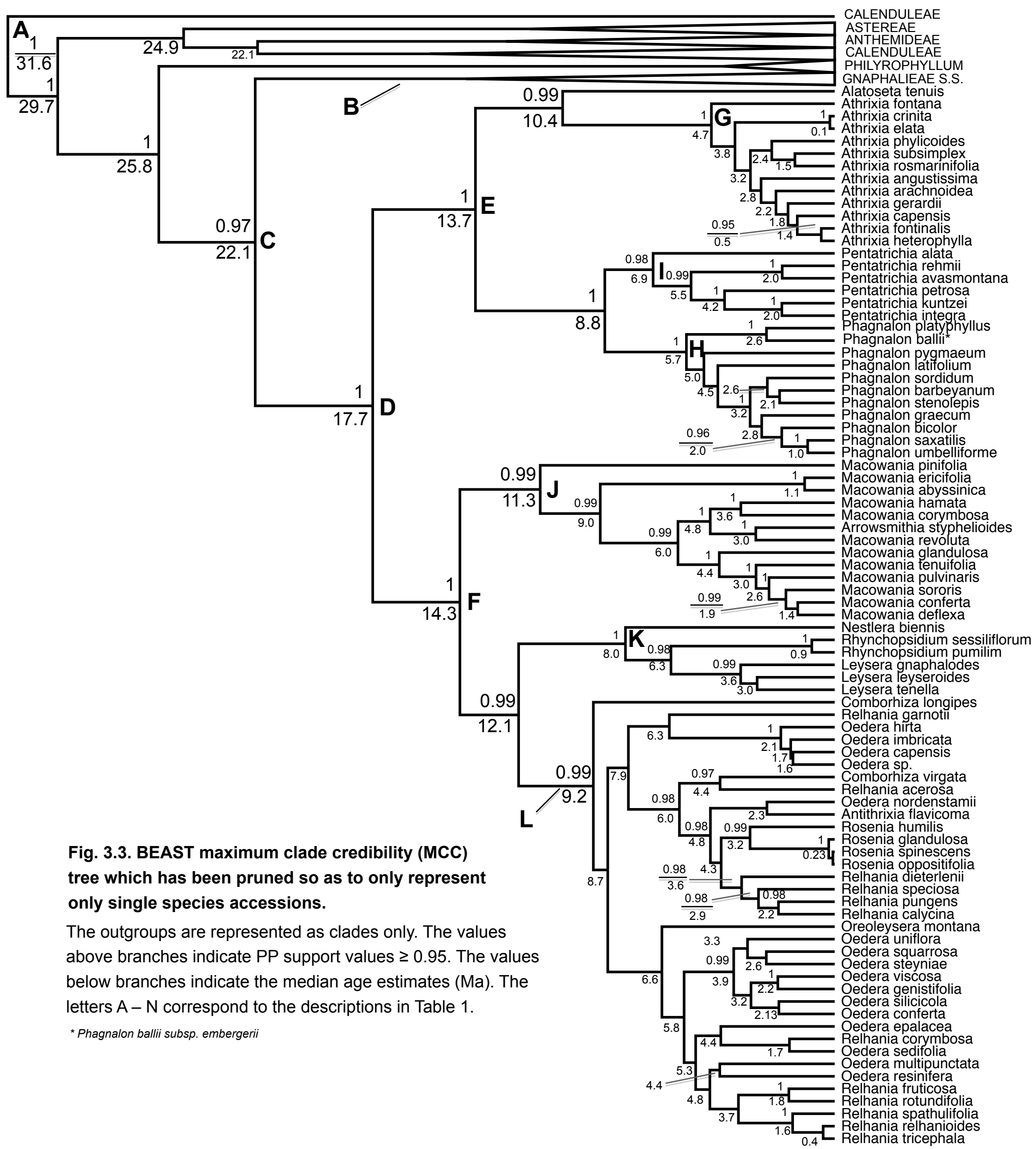



**Fig. 3.3. BEAST maximum clade credibility (MCC) tree which has been pruned so as to only represent only single species accessions.**

The outgroups are represented as clades only. The values above branches indicate PP support values ≥ 0.95. The values below branches indicate the median age estimates (Ma). The letters A – N correspond to the descriptions in Table 1.

** Phagnalon ballii subsp. embergerii*

## Migration scenarios and ancestral area reconstructions

**Table 3.2. The – *ln* likelihood scores of the nine migration scenarios presented, arranged from highest – *ln* likelihood score to lowest.** Models which achieved a score within the two – *ln* likelihood confidence interval are indicated with *. The third column, describing the geographic origin of the root node, depicts the area reconstructed on the upper branch / lower branch of the node. Reconstructed areas are provided until their summed probability is ≥ 0.50. The final column indicates the probability associated with the ancestral area reconstructed. The circum-Mediterranean region is shortened to "Med". Refer to Appendix 1 for the – *ln*L scores of other models tested.

| Scenario | – *ln* likelihood value | Geographic Origin of the root node | P |
|---|---|---|---|
| viii* | – 111.219 | GCFR / GCFR + Drakensberg | 0.47 |
| | | GCFR / GCFR | 0.19 |
| vii* | – 112.476 | Med + GCFR / GCFR | 0.67 |
| ix* | – 113.147 | GCFR / GCFR + Drakensberg | 0.43 |
| | | GCFR / GCFR | 0.16 |
| ii | – 116.049 | GCFR / GCFR + Drakensberg | 0.43 |
| | | GCFR / GCFR | 0.15 |
| i | – 117.953 | Drakensberg / GCFR + Drakensberg | 0.29 |
| | | GCFR / GCFR + Drakensberg | 0.24 |
| v | – 118.521 | Med + Drakensberg / Drakensberg | 0.27 |
| | | Med + GCFR / GCFR | 0.25 |
| vi | – 118.66 | Drakensberg / GCFR + Drakensberg | 0.34 |
| | | Drakensberg / Drakensberg | 0.19 |
| iv | – 123.413 | Med + Drakensberg / Drakensberg | 0.54 |
| iii | – 130.686 | East Africa + Drakensberg / Drakensberg | 0.99 |

## Testing alternative migration scenarios:

Several migration routes received consistently poor support in the dispersal scenario comparisons. For instance, the performance of models (scenarios) which allow for migration between D/EA and M was consistently poor when evaluated in terms of – *ln* likelihood (– *ln*L) scores (e.g. Fig. 3.2: i, iv, v, vi and Appendix 1). This suggests that the Afrotemperate and arid tracks comprise distinct

dispersal routes. The performance of models disallowing migration between D and EA was also consistently poor (e.g. Fig. 3.2: iii and Appendix 1), indicating the importance of D as a stepping-stone between the Cape region and the East African highlands. Those models disallowing migration between C and K and between C and D were interpreted by Lagrange as invalid operations (negative infinity values), suggesting that disallowing the exchange of species between these regions results in very poor model performance.

Of the nine migration scenarios presented (Fig. 3.2), scenario (viii) had the highest likelihood (lowest – *ln*L score: Table 3.2). This model requires independent dispersal routes from southern Africa to the circum-Mediterranean region, and from southern Africa to mesic Afrotemperate East Africa, allowing movement along the arid track (C – M) to be either direct or indirect (via K) but forcing dispersal along the Afrotemperate track (C – EA) to pass through D. A related model which differs only in allowing direct migration between C and EA performed less well (Appendix 1), implying that D plays an important role as a stepping-stone along the Afrotemperate migration route. The model with the second highest likelihood (vii) is similar to (viii), differing only in disallowing direct dispersal between C and M. This model fell within two – *ln*L units of the optimal model, implying similar performance (Edwards 1992; Ree & Smith 2008). While this appears to identify direct dispersal between C and M as unimportant, the inference of an improbably widespread ancestor (C+M) in scenario (vii) argues the opposite (see Table 3.2). Under this model, the disallowance of dispersal between C and M forces all nodes from the *Phagnalon* crown node to the root node to be interpreted as occurring in both C and M. The implication is that migration between C and M is very important. The scenario having the third highest likelihood (ix) is identical to (viii), differing only in allowing migration between D and K. Because this scenario also falls within two – *ln*L units of the optimal scenario, the implication is that dispersal between D and K is significant.

## Ancestral area reconstruction

The results of the ancestral area reconstruction generated under the optimal model is presented in Fig. 3.4. The *Relhania* clade is reconstructed as having an origin in southern Africa, the daughter lineage splitting into one descendant in C alone and the other into C+D (P=0.47). While the probability of this inference is modest, the second most-likely state also reconstructs to C (P=0.19). This suggests reasonable evidence for an origin of the group in the GCFR, with subsequent dispersal into the sub-alpine regions of the Drakensberg. A breakdown of the principal northward dispersal patterns in Athrixiinae and Oederinae is as follows: The crown node of Athrixiinae maps to C, though the probability of this reconstruction is modest (P = 0.62). This node yields two daughter lineages, one (*Athrixia* + *Alatoseta*) mapping to C (P = 0.58), the other (*Pentatrichia* + *Phagnalon*) to C+M (P = 0.98). Taken together, these reconstructions indicate a range expansion from C to M, via the arid track. This event is dated to 8.9 Ma (95% HPD: 6.0 – 12.0), placing it in the Late Miocene.From this node, one daughter lineage gives rise to a strictly circum-Mediterranean clade (*Phagnalon:* PP=1.0) while the other gives rise to *Pentatrichia* with ancestry in C+D (PP=1.0), representing a dispersal into D, at 7 Ma (95% HPD: 4.4 – 9.8). The following node, representing the ancestor of *Pentatrichia* excluding *P. alata,* represents a migration into K (P=1.0) followed by a range contraction to C alone (PP=1.0).

The ancestor of *Athrixia* reconstructs to C+D (P=0.72), representing a migration into D. This widespread distribution between both C and D is inferred to have persisted in the ancestor of the Cape-centred *A. crinita* and Drakensberg *A. elata* (P=1.0). One daughter lineage, however, is inferred to have experienced a range contraction to D (P=0.83). From this lineage, a daughter species occurs in EA+D (P=0.96) indicating migration along the Afrotemperate track, giving rise to a sister relationship between one species which occurs throughout these two regions (*A. phylicoides*), and another two sister species which are endemic to EA (*A. rosmarinifolia* and *A. sub-*

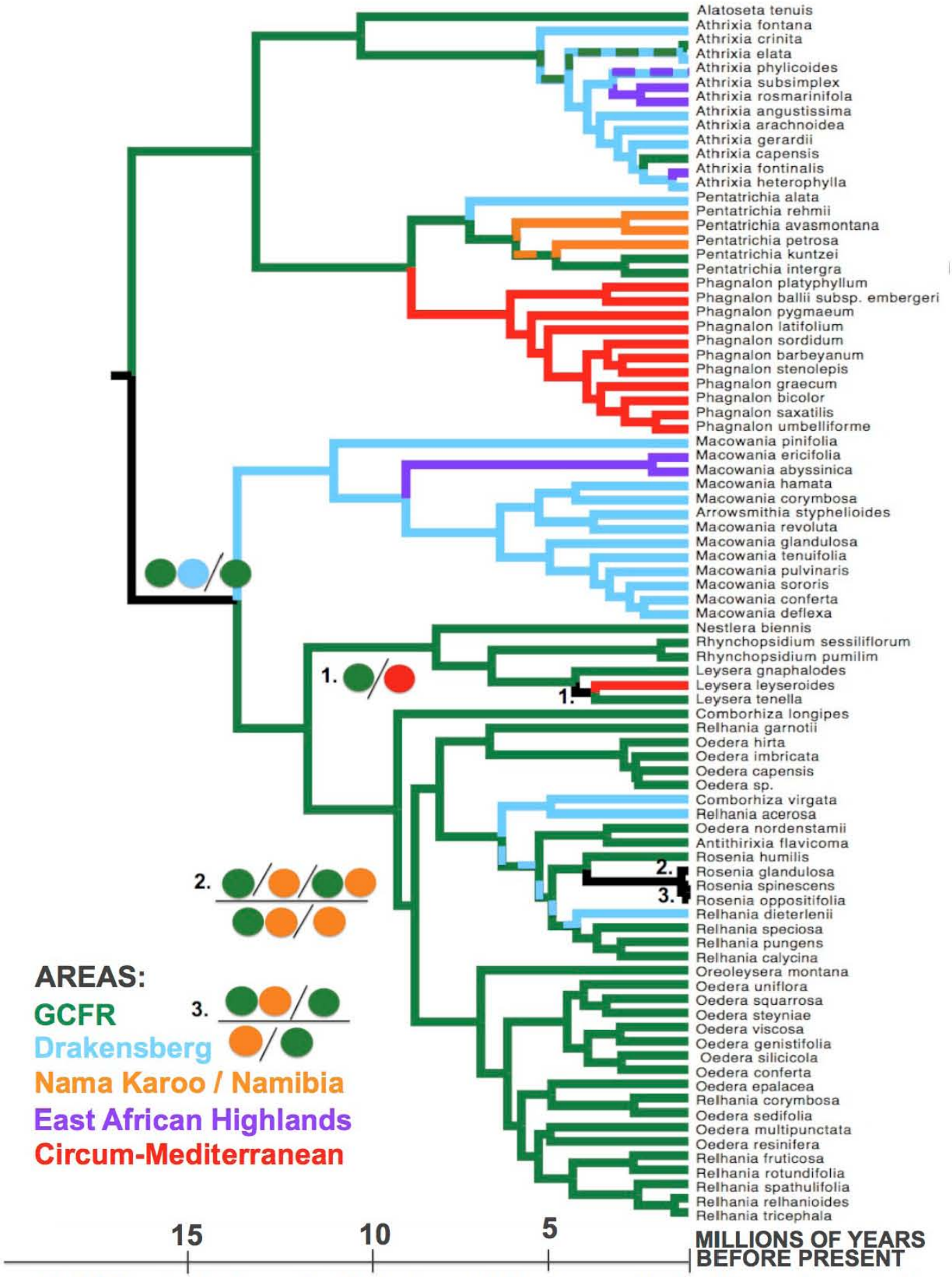



**Fig. 3.4. Tree showing the results of the best-supported Lagrange model (viii). All mapped reconstructions have marginal proportional likelihood (pL) > 0.50. Where no reconstruction has pL > 0.50 (black lines), the most probable reconstructions have been indicated (circles) whose summed pL is > 0.50.**

*simplex*). This places the migration to East Africa in the Middle Pliocene (3.4 Ma, 95% HPD: 1.1 – 4.1). The remaining daughter lineage is inferred to have occurred only in D (P=0.99), as do the following two nodes. A range contraction to C (P=1.0) then occurs with *A. capensis* suggesting a back-colonisation from D to C, and a migration from D to EA occurs in *A. fontinalis* (P=1.0) in the Late Pleistocene, indicating further Afrotemperate stepping-stone support.

The ancestor of Oederinae represents a daughter lineage split into C+D (P=0.85), with one daughter lineage then reconstructing to D alone (P=0.71), representing the stem node of *Macowania* at 11.3 Ma (95% HPD: 7.4 – 15.5), and the other reconstructing to C (P=0.94), representing a largely GCFR-centred group. Within *Macowania,* the next node reconstructs a split into EA+D (P=1.0), supporting an eastern Afrotemperate range expansion at 9.1 Ma (95% HPD: 5.8 – 13). In Oederinae, there has been a single migration from C to M along the arid track, in *Leysera leyseroides* (P=0.99) at 3 Ma (95% HPD: 1.2 – 5.0). Within Oederinae *sensu stricto*, all ancestors are reconstructed to C with high probabilities, indicating that this is a strongly GCFR-centred clade. There has, however, been an additional dispersal from C to D, with the two sister species *C. virgata* and *R. acerosa* (P=0.99) at 6.1 Ma (95% HPD: 3.6 – 8.5). A further expansion into the greater Drakensberg region occurs in *R. pungens* (not indicated on Fig. 3.4), but this is to lower elevations. A further range expansion from C into K occurs in *Rosenia* (PP=0.91).

### Habitat assignment and ancestral state reconstruction

The ancestral reconstruction of annual precipitation is presented in Fig. 3.5. The ancestral rainfall regime for the *Relhania* clade is inferred to be one of intermediate rainfall (593 mm). With the exception of the two annual lineages (*Alatoseta* and the "short-lived" clade) which adopt a xeric niche during the Miocene, all transitions to more xeric conditions occur in the Pliocene. This is particularly evident in Oederinae *sensu stricto* in *Rosenia, Oedera nordenstamii, O. silicicola* and

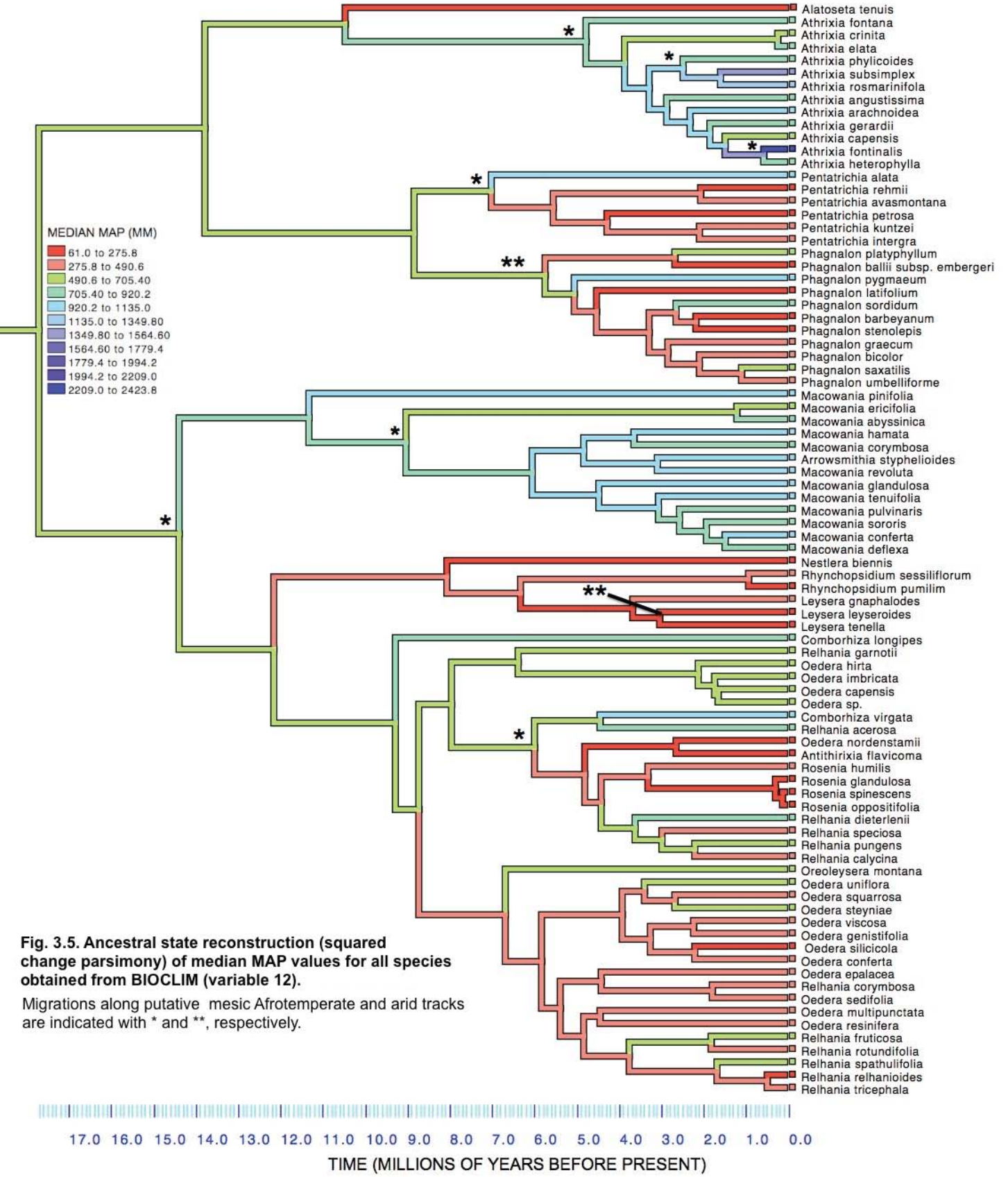



**Fig. 3.5. Ancestral state reconstruction (squared change parsimony) of median MAP values for all species obtained from BIOCLIM (variable 12).**

Migrations along putative mesic Afrotemperate and arid tracks are indicated with * and **, respectively.

*Antithrixia flavicoma.* All inferred transitions to a moister (Afrotemperate) niche also occur in the Pliocene, with the exception of the earliest-diverging lineage of *Macowania* (*M. pinifolia*) which occupied its sub-alpine Afrotemperate niche during the Miocene. *Athrixia* and *Macowania* both stand out as relatively mesic lineages (indicated in Fig. 3.5 by shades of green, blue and purple), all species having mean MAP habitat estimates of above 600 mm, and several having estimates of MAP greater than 1000 mm (e.g. 2209 mm in *Athrixia fontinalis*). Most of the remaining lineages have habitat MAP estimates of less than 500 mm (indicated in Fig. 3.5 by shades of red and pink), with several clades containing members with values below 400 mm. Based on the limited species sampled, *Phagnalon* appears to occupy a more diverse range of moisture niches, including highly mesic (1016 mm) and highly arid (61 mm).

## Discussion

Despite poor topological support within a few genera, the BEAST analysis produced a tree which is generally well-supported. In the context of this tree, the ancestral area reconstructions yield an inference of a South African origin for the *Relhania* clade, most likely to the GCFR. A southern origin has also been found for several other lineages with similar pan-African distributions (e.g. Bellstedt et al. 2012; Coleman et al. 2003; del Hoyo et al. 2009; Galley et al. 2007; Richardson et al. 2001), and has also previously been inferred for the *Relhania* clade, though this was done on the basis of a rather limited species sample (Bergh & Linder 2009). In the present study, the root node of the *Relhania* clade is (relatively) unambiguously dated to the Middle Miocene, with error bars extending from the Early Miocene to Middle Miocene (Table 3.1). Radiations in other lineages in the GCFR were initiated at this time too, some examples include *Indigofera* (Schrire et al. 2003), *Ehrharta* (Verboom et al. 2003), *Muraltia* clade II (Forest et al. 2007), *Oxalis* and *Melianthus* (Verboom et al. 2009); these being linked to newly available summer-arid habitats.

The root node of the *Relhania* clade reconstructs to a region most likely encompassing the GCFR, with subsequent diversification into surrounding arid regions and high-moisture zone of the Drakensberg. There is, however, reasonable uncertainty regarding the state estimated at the most probable and second-most probable reconstructions of the best-supported model (0.48 and 0.19 respectively). Dated at 18 Ma (95% HPD: 14.0 – 21.3 Ma), the origin of the *Relhania* clade is older than the onset of aridity in southwestern Africa, believed to have commenced from the Late Miocene (DuPont et al. 2011; Diekmann et al. 2003; Linder et al. 2003; Siesser 1980; Zachos et al. 2001). An origin in a relatively mesic climatic niche (593 mm) is inferred by the ancestral rainfall reconstruction. This constitutes a wetter niche than most of the arid-adapted lineages of the group and is more akin to the moisture niches of the modern Afrotemperate lineages. The ancestor of the clade is thus likely to have originated prior to, or alongside, the onset of aridification of southwestern Africa. It is believed that the Middle Miocene vegetation of southwestern Africa was wooded, sub-tropical with Afromontane forest dominated by *Podocarpus* (Coetzee 1978, 1983; DuPont et al. 2011). One possibility might be that ancestral lineages were contained within the mountainous areas of the Cape and/or the Karoo Escarpment. The Cape Fold Belt arose much earlier than the Drakensberg, at around 330 Ma (McCarthy & Rubridge 2005), while habitats along the Escarpment were probably available from the Early Miocene, due to tectonic uplift (Partridge & Maud 1987). Established montane lineages would have then been able to colonise the Escarpment and sub-alpine Drakensberg from that time. Most South African Afrotemperate *Relhania* clade lineages occupy C3 cool-temperate grasslands, and the ancestral Cape Fold Belt lineages would possibly have migrated eastwards along the C3-dominated Karoo Escarpment to the Drakensberg; this acting as a bridge linking the two regions, facilitating the migration of montane lineages (Mucina & Rutherford 2006), as summer-arid seasonality is estimated to have only become established later (DuPont et al. 2011).

The data presented allude to niche conservatism in dictating migration patterns in the *Relhania* clade, supporting previous suggestions that species are more likely to track their ecological niche than adapt to a novel one (Crisp et al. 2009; Kozak & Wiens 2006; Wiens 2004; Wiens et al. 2010). Dispersals along both the arid and Afrotemperate tracks are identified in this study. There is evidence for the distinctness of these tracks; in the best-supported reconstructions, a mesic East African connection for arid-adapted lineages in the north and south was inferred to be far less likely than a direct arid-arid connection, and similarly, Afrotemperate lineages do not connect via arid southern Africa or the circum-Mediterranean. The ancestral state reconstruction did not infer any track dispersal events to be associated with great changes in moisture regime; disjunct lineages instead appear to disperse along tracks which conform to a more-or-less uniform moisture regime. Modern species also tend to inhabit very narrow distributions, providing further circumstantial evidence for niche conservatism being important in maintaining species isolation through local differentiation.

The adoption of an annual or otherwise short life-history in the highly arid lineages of *Alatoseta* and the "short-lived" clade probably evolved as a strategy for surviving periods of aridity (Van Rooyen 1999). Similarly, a group of perennial taxa in Oederinae *sensu stricto* have infiltrated highly arid regions associated with the Succulent and Nama Karoo Biomes, indicating an ability to evolve high aridity tolerance in the *Relhania* clade. There has also been at least one colonisation of the highly mesic Drakensberg alpine zone in Oederinae *sensu stricto.* A similar pattern is evident in *Phagnalon* where species have infiltrated both mesic and arid habitats; both Oederinae and *Phagnalon* inhabit a predominantly mediterranean-type climate.

The best-fit model supports independent migrations along transient corridors of more-or-less continuous arid habitat (arid track) since the Miocene. An arid track migration scenario is especially

reasonable if one considers that there may have once been contiguous patches of suitable habitat, or even patches separated only by short intervals of unsuitable habitat, which still allow gene flow between populations. The two migrations occur in the Late Miocene (*Phagnalon*) and in the Pliocene (*L. leyseroides*). Thus, the timing of both events are broadly congruent with putative arid track migrations estimated in several other lineages, and also coincide with estimated timing of aridification across Africa. The earlier date (8.8. Ma, 95% HPD: 6.0 – 11.9) closely matches Late Miocene arid track disjunctions in *Androcymbium* estimated at 8.3 and 8.5 Ma (Caujape-Castells et al. 2001; del Hoyo et al. 2009), the weevil genera *Rhinusa* and *Gymetron* at 8.5 Ma (Hernandez-Vera et al. 2013) and *Thamnosma* at 8.5 Ma (Thiv et al. 2011). They also coincide with previous estimations for the existence of an arid track during the Late Miocene (Balinsky 1962; Van Zinderen Bakker 1969, 1975; Verdcourt 1969). The later date (3 Ma, 95% HPD 1.2 – 5.0) coincides broadly with the inferred Pliocene migrations of *Zygophylloideae* (Bellstedt et al. 2012). Conditions on the African continent have become progressively drier since the Middle Miocene, with periods of heightened aridity in the Late Miocene (ca. 6 Ma) and the Plio-Pleistocene boundary (ca. 2 Ma; Bobe 2006; Burke & Gunnell 2008; deMenocal 1995, 2004). Climate model experiments based on fossils and marine sediments suggest intense periods of aridity in sub-tropical Africa around 1.0, 1.7 and 2.8 Ma (deMenocal 2004), in the region of Namibia between 3.4 and 3.0 Ma and again between 2.2 and 1.8 Ma (DuPont et al. 2005; DuPont 2006), and in the Horn of Africa at 0.9 Ma and 3.2 Ma (Trauth et al. 2009). Models also imply that Late Miocene (ca. 8 Ma) tectonism and rift-flank uplift in the East African rift system drove aridification in the lower regions of the rift system which corresponds to the eastern extent of the arid track (Sepulchre et al. 2006). Along the northern reaches of the track, the Mediterranean Basin is also believed to have become increasingly arid around 9 – 8 Ma (Ivanov et al. 2002; Fortelius et al. 2006; Van Dam 2006), with a mediterranean-type climate being firmly established by 3 Ma (Suc 1984; Thompson 2005).

The source area for both of these colonisations of the circum-Mediterranean is inferred to be the GCFR rather than the region characterised as K (the Karoo-Namib arid area), despite the use of a dispersal scenario that allowed migration to M to proceed either directly from the GCFR, or via K as an intermediate. This suggests that the Namib region has not played an important role as stepping-stone for the northwards migration of these lineages. While the Namib region appears to have been entered twice, in neither instance has it acted as a stepping-stone to the circum-Mediterranean. No relictual populations exist at intervals along the track and while the pappus in the *Relhania* clade is weakly developed and shows no indication of epizoochory, the possibility of long-distance dispersal as a corollary cannot be discounted by the data, as dispersal across seemingly impossible distances does occur (e.g. as suggested in Coleman et al. 2003; Thiv et al. 2011). Nevertheless, both inferred arid-track migrations in the *Relhania* clade occur in lineages with close relatives in K. *Leysera tenella*, sister to *L. leyseroides*, occurs in both Namaqualand and the Nama Karoo region. The sister clade to *Phagnalon* is *Pentatrichia*, with most of its members in arid Namibia. The summer–rainfall arid regions represented as K in the present study extend northwards into Africa and may in the past have served as the source area for arid-track migrations, with subsequent signals of this connection being lost.

Several instances of migrations between the GCFR, Drakensberg and East African Highlands are also identified by the best-fit model, and the best-supported models all facilitate D as an Afrotemperate stepping-stone. The best-fit model disallows direct migration between the GCFR and eastern Afrotemperate region, which accords with the findings of Galley et al. (2007) that the direct migration of taxa from the GCFR to eastern Africa is rare. Also, the most poorly supported models (iii and Appendix 1) restrict the stepping-stone migration of Afrotemperate lineages via the Drakensberg. Middle Miocene tectonic uplift along the eastern Escarpment of southern Africa likely created sub-alpine habitats, and more pronounced tectonic uplift of around 900 m is estimated

to have occurred approximately 5 Ma (Davies et al. 1977; Partridge & Maud 1987; Partridge 1998; Partridge & Brink 1967; Watson & Williams 1985), creating a high-altitude Afrotemperate zone available for colonisation. There are at least four independent instances of migration from the GCFR into Drakensberg in the *Relhania* clade, most occurring subsequent to the availability of high-altitude Afrotemperate habitats offered by uplift of the Drakensberg (< 5 Ma). One of the northwards migrations occurs sometime between 14.5 Ma and 11.3 Ma, representing the Drakensberg migration of the ancestor of *M. pinifolia.* However, this early dispersal was probably to somewhat lower-elevation, sub-alpine terrain (prior to Pliocene uplift) as inferred on the basis of the present day niche of *M. pinifolia.* Other studies have also reported numerous migrations between the two regions in genera such as *Disa, Euryops, Restio* and *Pentaschistis* at times following Pliocene uplift as well as during the Miocene (e.g. Devos et al. 2010; Galley et al. 2007). One migration (*A. capensis*) is unusual in being southwards to the GCFR. While Galley et al. (2007) find evidence for southwards migrations, the overwhelming pattern is a northwards one. There are also instances of Drakensberg – East Africa migrations, two in *Athrixia* and one in *Macowania*. Rift-flank uplift is believed to have commenced in its northern extent in the region of Ethiopia from 11 Ma, giving rise to the typical tripod-shaped rifts forming the Suez canal, the rift Valley and the Gulf of Aden. Rifting commenced southwards forming the high-altitude zones of Malawi and Kenya, at later intervals during the late Miocene and Pliocene (Chorowics 2005; Flannery & Rosendahl 1990; Wolfenden et al. 2004). All migrations northwards into these regions are inferred to have commenced only after 11 Ma, suggesting that northwards migration happened only when high-altitude Afrotemperate habitats became available, the Drakensberg playing an important role as an intermediary between the Cape and East Africa. The high regions of Chimanimani, Kenya and Malawi also possibly acted as stepping-stones to the highlands of Ethiopia and Yemen. Though the pappus of *Athrixia* and *Macowania* may not be especially designed for long-distance dispersal, the intervals (stepping-stones) at which taxa would have

dispersed along are relatively contiguous (e.g. see Fig. 3.1), especially if favourable climate lowered the elevation of the mesic Afrotemperate zone. The Drakensberg also harbours the only significant radiation of Oederinae species outside the GCFR, in *Macowania.* This genus experiences a minor radiation in the Drakensberg, also likely in response to the creation of new alpine habitats through Pliocene tectonism: an idea that is further explored in Chapter Four.

## Conclusion

The evolution of the *Relhania* clade appears to be strongly linked to climatic and tectonic changes in Africa and the Mediterranean since the Miocene. Separate Afrotemperate and arid corridors and/or stepping-stones have facilitated trans-African migrations to regions of similar habitat on opposing sides of the continent. The severing of the arid track as a result of phases of aridity from the Late to Middle Miocene culminated in the geographical isolation of species, where they have differentiated to form distinct entities in the absence of gene flow. In terms of the Afrotemperate track, the role of Afrotemperate peaks, especially the Drakensberg, as stepping-stones for the transport of taxa northwards is found to be an unequivocally important biogeographic phenomenon. Despite a significant centre of diversity in the circum-Mediterranean region, the clade is inferred to have a southern African origin, most likely in the GCFR with subsequent dispersal into the greater Drakensberg. Most lineages experienced a shift to slightly more arid habitats, although there are notable shifts towards higher-rainfall habitats in *Athrixia* and *Macowania.* Using habitat information to complement biogeographic reconstructions allows for a more nuanced examination of biogeographic history. In the *Relhania* clade, the reconstruction of moisture niches in tandem with inference of the phylogenetic placement and age of dispersal events has allowed for the identification of independent arid-adapted and Afrotemperate-adapted migrations.

The Drakensberg has evidently played a significant role in the evolution of the *Relhania* clade, as

lineages within the clade (with the notable exception of *Phagnalon*) all have strong associations with the region. In Oederinae, there is at least one instance of a more recent migration into the Drakensberg from the GCFR. Overall, the majority of migrations out of the GCFR in both subclades have been into the greater Drakensberg region. *Macowania* has experienced a minor radiation in the Drakensberg probably in association with Pliocene tectonism. Uplift at this time is believed to have triggered landscape erosion and rejuvenation (Partridge & Maud 1987, 2000), and possibly, promoted speciation through vicariance and adaptive divergence. Chapter Four will build on this theme.

# Chapter Four: Erosive processes after tectonic uplift stimulate vicariant and adaptive speciation: evolution in the Drakensberg near-endemic genus *Macowania*

## Introduction

The formation of major mountain chains by tectonic uplift has stimulated plant diversification in many parts of the world, and the resulting diversity may be spectacular. Documented examples include the Andes (Burnham & Graham 1999; Hughes & Eastwood 2006; Simpson 1975), the Mexican Sierra Madre (Bryson et al. 2011, 2012, 2012b) and the Himalayas (Xu et al. 2010; Yang et al. 2012). For example the northern Andes harbours some 45,000 plant species, 44% of which are endemic (Myers et al. 2000), with the northern Andean páramos topping this with 60% endemism (Luteyn et al. 1999). However, the specific mechanisms by which uplift may influence species divergence have seldom been explicitly explored. In this chapter, the mechanisms underlying speciation following uplift of a diverse South African mountain system is explored.

In southern Africa, Pliocene tectonic uplift played a major role in creating the geomorphically diverse Drakensberg range (King & King 1958; Partridge & Maud 1987, 2000; Partridge 1998). The Drakensberg constitutes the higher, eastern façade of the Great Escarpment of South Africa (Fig. 4.1). In terms of both height and endemic plant diversity, the Drakensberg is more modest than the Andes, with a maximum altitude of 3,482 m and the core area hosting ca. 2,520 species or subspecies of flowering plants, of which 16% are endemic (Carbutt & Edwards 2004). Nevertheless, the Drakensberg region is one of three centres of Afrotemperate endemism, and appears to have been an important stepping stone between the centre in the East African Highlands and that in the highly diverse Cape region (see Chapter Three).

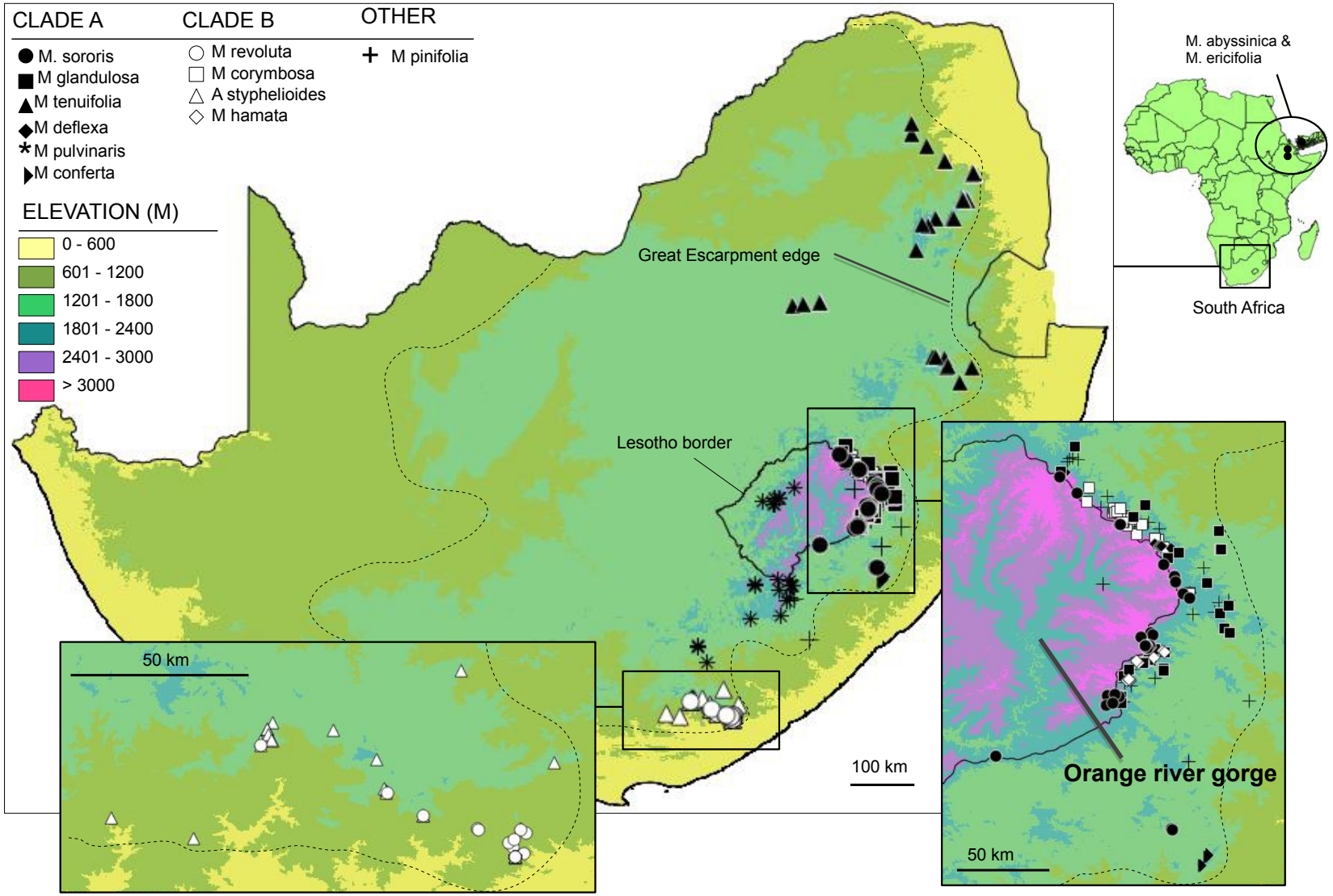



**Fig. 4.1 Distribution map of *Macowania* and *Arrowsmithia.*** Clade A species are indicated by black symbols, and clade B species by white symbols. The dotted line represents the Escarpment edge. The disjunct distributions of *M. abyssinica* and *M. ericifolia* in the East African Highlands and Yemen are indicated at the top right of the figure. Insets of selected parts of the distribution in South Africa are provided for small areas that house multiple species.

Tectonic uplift might stimulate speciation in two principal ways. First, pronounced uplift will produce a novel, high-altitude adaptive zone which may serve as an arena for adaptive radiation (*sensu* Simpson 1975). The scale of radiation that occurs within such an adaptive zone will ultimately depend on the heterogeneity of habitats on offer, but this is likely to be high owing to the effectiveness of tectonism and subsequent erosion in generating situations that vary in terms of altitude, aspect, slope, geology, soil type, microclimate and moisture regime (e.g. stream beds versus rocky ridges). Underlying geological heterogeneity will increase the overall habitat diversity by allowing for specialisation to different soil types as they are exposed or created by erosion; it will also result in a more complex landscape due to differences in erodibility. Tectonism may also promote species radiation indirectly, by the stimulation of large-scale erosion resulting from increased river gradients. Removal of increasing portions of the landscape will disrupt the original land surface, potentially fragmenting species ranges. Given time, populations separated by erosion gaps will diverge as a consequence of both adaptive divergence and neutral processes (drift), the latter being more important where the selective differentials between populations are low and/or population size is small with limited gene flow (Lande 1976). An example of neutral divergence following erosion-mediated habitat fragmentation is the divergence of a montane lizard genus (*Phrynosoma*) in the Sierra Madre of Mexico, where canyon formation followed tectonic uplift (Bryson et al. 2011, 2012, 2012b). Although the potential importance of non-adaptive divergence as a driver of speciation (non-ecological speciation) is gaining recognition (Kozak et al. 2009; Rundell & Price 2009; Wiens 2004), the long-standing emphasis on adaptive divergence as the sole agent remains pervasive (McKinnon et al. 2009; Orr & Smith 1967; Schluter 2000, 2009), and it is difficult to prove the absence of any form of adaptation.

In principle, post-uplift species accumulation is likely to occur as a consequence of both adaptive and non-adaptive processes. If this is true, tectonic processes and subsequent erosion have likely

been important as stimuli for both adaptive and non-adaptive diversification in the Drakensberg. One of the predictions of this hypothesis is that the bulk of diversification in endemic lineages should be associated with (or occur soon after) episodes of major tectonic activity. Also, where non-adaptive processes have been important, we expect to find strong signatures of allopatric speciation, paired with limited functional divergence. A brief overview of the geological history of the region reveals high probability of erosion-mediated range fragmentation. Composed of large blocks of sedimentary and volcanic rocks (primarily the soft 'cave' sandstones of the Clarens group topped by the younger and erosion-resistant basalts and dolerites of the Drakensberg group) which have been deposited over the past 200 Ma, the highly incised contemporary landscape of the Drakensberg is a product of erosion associated with cyclical uplift throughout the Cenozoic. Following the separation of Africa from the rest of Gondwanaland at about 184 Ma, the eastern half of South Africa experienced several cycles of uplift. The two most recent cycles of uplift, during the Miocene (150 – 300 m) and Pliocene (600 – 900 m: Davies et al. 1977; Partridge & Maud 1987; Partridge 1998; Partridge & Brink 1967; Watson & Williams 1985), are each thought to have stimulated renewed erosion of the Great Escarpment (King & King 1959; Partridge & Maud 1987, 2000). Over time, the resulting Escarpment edge, originally a uniform plateau extending to nearly the present-day coastline (King & King 1959), is hypothesised by some authors (King & King 1959; Partridge & Maud 1987, 2000) to have eroded back towards the interior of the country, simultaneously being incised by a series of deep drainage gulleys. In the absence of historical volcanism, the contemporary deeply-dissected and geologically heterogeneous Drakensberg landscape is thus a product of landscape erosion.

Dispersal both to and from the Cape via the Drakensberg appears to have been an important factor in the genesis of Afrotemperate plant diversity. Regional floras are assembled via both immigration and *in situ* diversification. Most Drakensberg-endemic lineages studied to date appear to be the

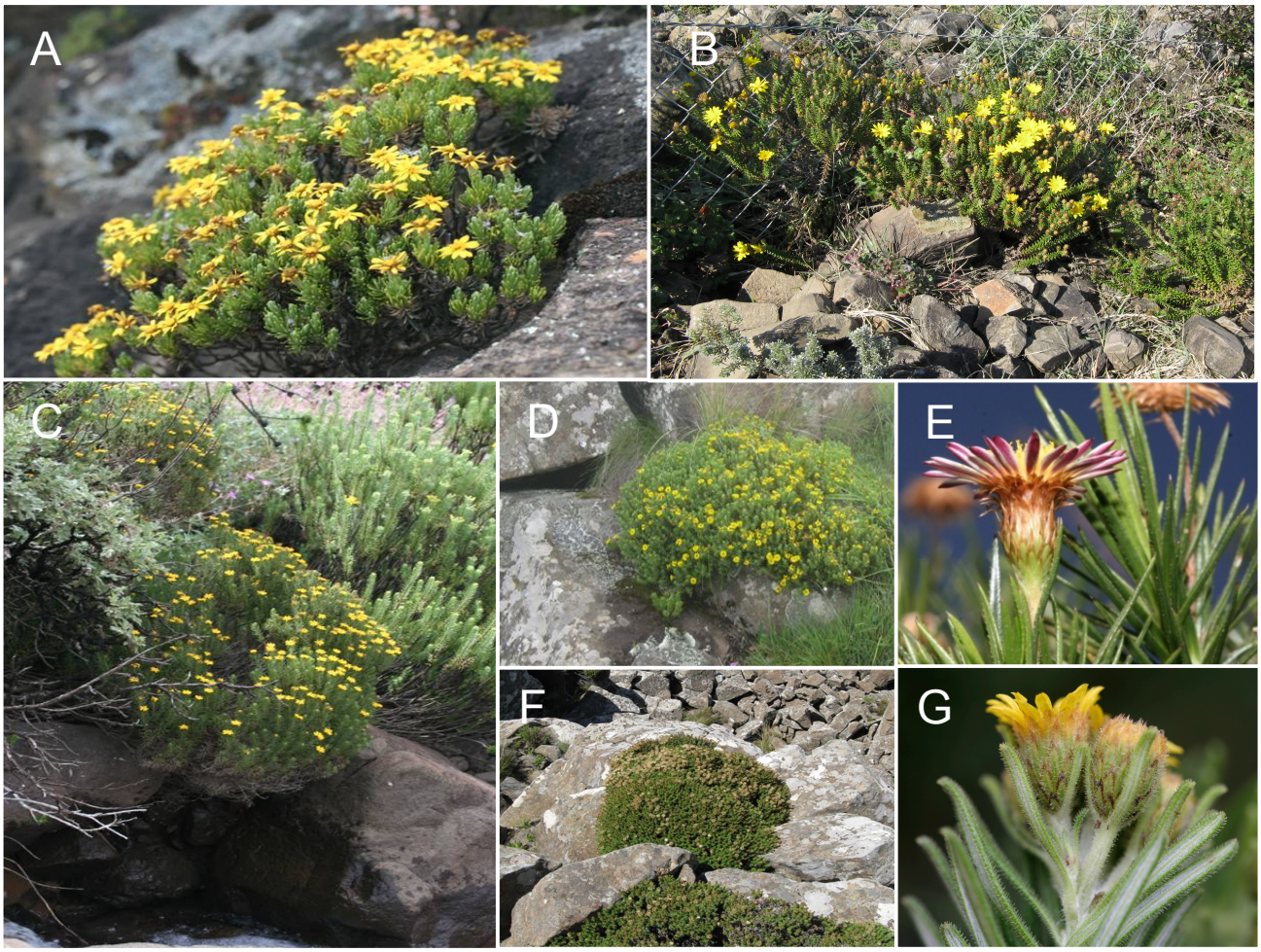



**Figure 4.2. Habitat, habit and morphology of selected study species.**
A: *M. pulvinaris* (clade A). B: *Arrowsmithia styphelioides* (clade B). C: *M. hamata,* overhanging a stream (clade B). D: *M. conferta* with cushioned growth form (clade A). E: *M. pinifolia*, unusual in Macowania due to its pin*k* ray florets and non-revolute leaves. F: Habitat of *M. sororis* cushioned among dolerite rocks/boulders (clade A). G: Typical *Macowania* inflorescence and leaves of *M. corymbosa* (clade A), also showing the dark-edged bracts shared by *R. revoluta* and *M. corymbosa.* Photos by C. Mckune and N.G. Bergh.

result of repeated independent dispersal into the region, rather than *in situ* diversification (Galley et al. 2007). Evidence for this lies in the low number of endemic species per genus. Of the 37 genera that contribute more than three endemic species to the Drakensberg Alpine Centre (DAC: the central, highest-lying part of the Drakensberg range [Killick 1994; Van Wyk & Smith 2001]), only two have more than 12 endemics and the average is 6 endemic species (Carbutt & Edwards 2006).

Although the scale of *in situ* radiation varies among Drakensberg plant lineages (Galley et al. 2007) it appears for the most part to be modest, which may reflect both a youthful colonisation history and the small scale of the region. The daisy family Asteraceae has been the most successful angiosperm coloniser of the Drakensberg, with several genera contributing high numbers of endemic species to the DAC (*Helichrysum*: 29; *Senecio*: 22; *Euryops*: 7 [Killick 1994]).

*Macowania* has five species strictly endemic to the DAC, but eleven species (including *Arrowsmithia*) endemic to the greater Drakensberg area (including the escarpment of the Eastern Cape, KwaZulu-Natal and Mpumalanga provinces). The two non-Drakensberg members occupy the East African Afrotemperate Highlands of Ethiopia, Djibouti, Eritrea and Yemen (Fig. 4.1). This makes it comparatively species-rich, and an excellent system for exploring the impact of landscape evolution on speciation in the greater Drakensberg area. The South African species of *Macowania* are essentially restricted to high-elevation habitats, the majority favouring rocky environments along or immediately below the Drakensberg scarp edge. Here they inhabit a diversity of substrata or geologies, and some degree of substrate-specificity is apparent. Three species are unusual in preferring riparian or frequently-moist habitats.

In this chapter, the dated molecular phylogenetic hypothesis obtained in previous chapters is used in conjunction with distributional and morphological data to explore speciation pattern and process in *Macowania*. The monotypic *Arrowsmithia* is included as, despite its contrasting vegetative morphology, phylogenetic analysis reveals that the two genera are best synonymised (see Chapter Two). In view of the overwhelming association of *Macowania* with high-elevation habitats, one hypothesis is that the Drakensberg species of *Macowania* constitute a clade whose contemporary diversity is the product of a minor radiation associated with dramatic Pliocene uplift of the Drakensberg scarp. The discovery within *Macowania* of two principal clades, one restricted to

more-or-less uniform high-elevation rocky habitats (clade A) and the other occupying a broader array of ecological situations (clade B, occurring on rocky slopes, along streams and in seepages), indicates a potential role for both non-ecological and ecological speciation processes (Figs. 4.1 & 4.2). One speculation is that, whereas erosion-induced fragmentation of the scarp zone has resulted in a history of primarily vicariant speciation in clade A, speciation in clade B has been powered to a greater extent by adaptive divergence. To evaluate these ideas, several predictions, consistent with a vicariant speciation model, are tested: (i) the signatures of allopatric speciation should be stronger in clade A than in clade B; (ii) morphological divergence, used as a proxy for functional diversification, should be less pronounced in clade A than in clade B; and (iii) where related species have highly overlapping distributions, especially in clade B, morphological divergence should be higher. Molecular dates obtained in Chapter Three are used to evaluate the hypothesis that radiation of these clades closely followed recent Pliocene tectonic uplift.

# Methods

### Estimation of species relationships and divergence dates

The phylogenetic relationships of *Macowania* and *Arrowsmithia* are revealed and discussed in Chapter Two. The dated BEAST tree topology obtained in Chapter Three is trimmed and modified to represent only single species accessions of *Macowania* and *Arrowsmithia* and used to estimate the nested range overlap (see below).

### Range overlap

To measure pairwise range overlap, species' ranges were estimated by plotting point locality data and calculating convex hull polygons. The localities of all relevant specimens at five South African herbaria (NBG, BOL, PRE, PRU, NU), as well as field observations by the authors were geo-referenced as precisely as possible using 1: 50 000 topographic maps (Chief Directorate: Surveys &

Mapping, Mowbray, Cape Town) in an ArcMap 10 environment (ArcGIS Desktop 10 Service Pack 2: CA: ESRI), as well as Google Earth and Google Maps. Where recorded, GPS co-ordinates provided a precise indication of locality. Convex hull polygons (i.e. the polygon that would be created by placing a tightly-stretched elastic band around all the point localities for a species) were produced using the 'clusthr' command in the 'adehabit' package (Calenge 2006) in R 2.15.1 (R Development Core Team 2008). Convex hulls yield simplistic estimates of species' ranges, ignoring range discontinuities and irregularities in range boundaries. Nonetheless, they are likely to closely approximate the ranges of species that have compact, continuous distributions, as is typical for *Macowania* and *Arrowsmithia.* In addition, the fact that this method over-estimates the extent of geographic ranges renders it a conservative measure of allopatry, making it robust for this purpose. Since convex hulls are sensitive to spatial errors (Burgman & Fox 2003), every effort was made to check and correct specimen identifications and locality information, and doubtful localities were excluded. Nevertheless, for several taxa very few precise localities were available resulting in some observations that were deemed accurate only to the nearest 5,001 – 10,000 m, and the minimum number of locality points being six for the local endemic *M. deflexa*. Once the polygons were defined, range overlaps between species was calculated as per Fitzpatrick and Turelli (2006) using the dated tree topology.

### Testing for adaptive differentiation

Adaptive divergence in montane settings could be driven by a range of selective forces, acting alone or in combination (for example, specialisation to particular climatic or edaphic niches or to different pollinators). As a proxy for adaptive differentiation, a suite of vegetative and reproductive characters that were relatively uniform within species of the core *Macowania* clade, but able to fairly reliably differentiate amongst species, were identified. The rationale was that characters showing such patterns are likely to be under selection, or linked to other traits that are under

selection. The characters selected were capitulum length and width (relating to reproduction, including floral display, seed size and number and seed protection) and leaf length and width (shown to be strongly correlated with plant habitats [Yates et al. 2010]). Precision callipers were used to measure 20 specimens per species, choosing representatives from across the geographic range of each. Owing to limited numbers of herbarium specimens, fewer measurements were taken for the localised endemics *M. deflexa* (two specimens), and *M. conferta* (six), as well as for *M. hamata* (14) and *M. revoluta* (18). For each character, the measurements from all specimens of a species were averaged and input into a pairwise multivariate discriminate functions analysis (DFA), as implemented in R 2.15.1. Mahalanobis' (1936) distance, widely used in biological clustering, was then calculated between species. Mahananobis' distance uses both the mean and variance of the predictor variables, as well as the covariance matrix of the variables, thus taking advantage of the covariance among variables. By transforming measurements into standardised uncorrelated data which is used to estimate Euclidean distances, scale differences are taken into account when estimating distances.

# Results

### Phylogenetic relationships and divergence date estimates in *Macowania*

*Macowania* is confirmed as monophyletic by bootstrap, Bayesian and BEAST PP in Chapters Two and Three, subject to the inclusion of *A. styphelioides.* Relationships in *Macowania,* and especially within the 'core' clade (i.e. those endemic to the greater Drakensberg and excluding *M. pinifolia*), receive excellent support (see also Chapter Two). In terms of divergence dates, *Macowania pinifolia* diverges earliest with a median age of 11.4 Ma (95%HPD 7.4 – 15.5 Ma). The divergence of the East African *Macowania* species is dated at 9.1 Ma (95%HPD 5.8 – 13.0 Ma). Two primary subclades are identified in the 'core' clade (Fig. 4.3): clade A and clade B. The median crown age of the 'core' *Macowania* clade is dated to 6 Ma (95%HPD 3.8 – 8.6 Ma), which places it at the

Miocene-Pliocene boundary, with error bars extending from the latter part of the Late Miocene to the Pliocene (Fig. 4.3). The radiations of the two subclades (clade A and B) have synchronous ages and occur in the Pliocene (Fig. 4.3).

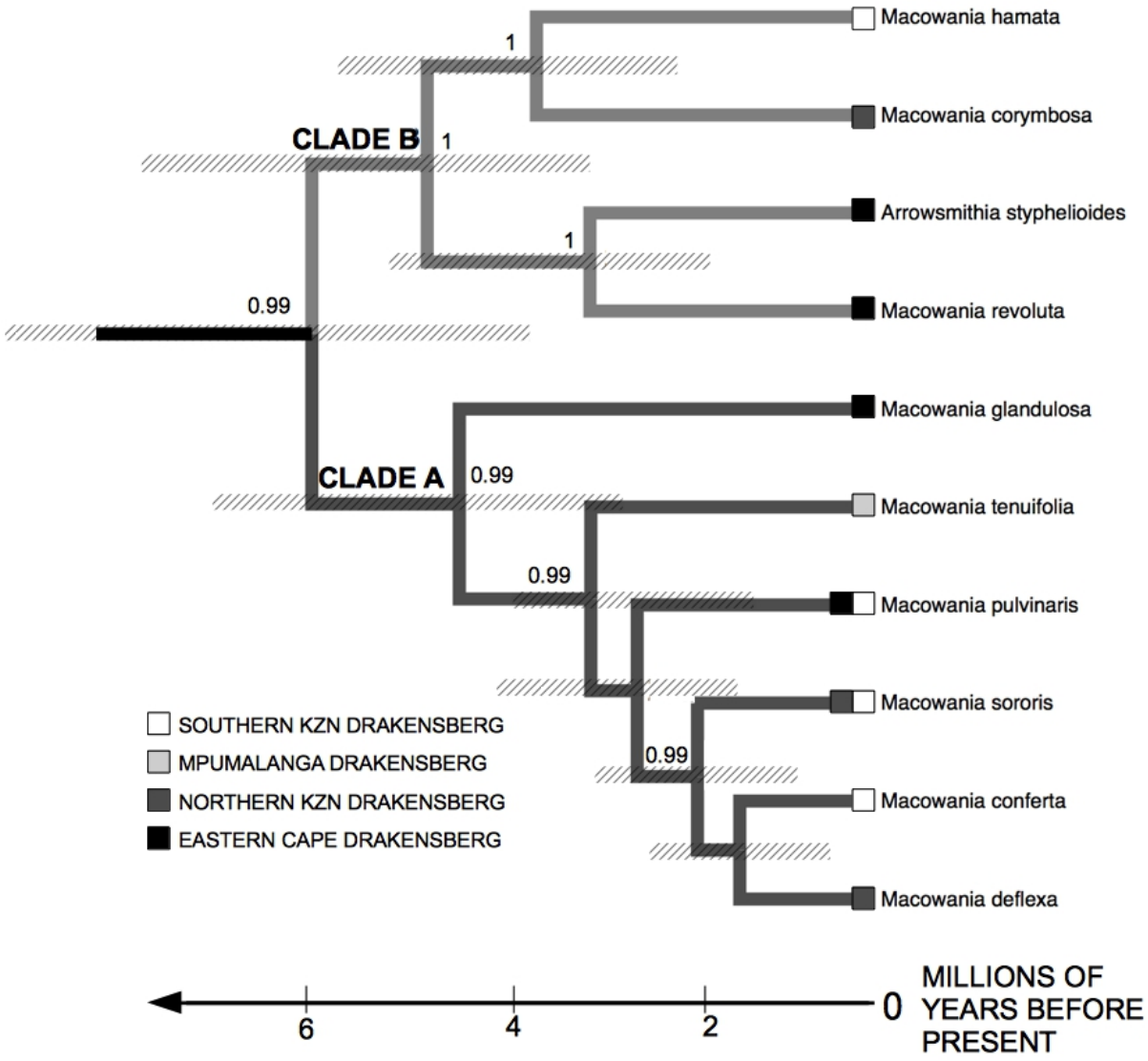



**Fig.. 4.3 'Core' *Macowania* clade phylogeny obtained from BEAST indicating membership and relationships within clades A and B.** BEAST PP values ≥ 0.95 are indicated at nodes, and the error bars represent the 95%HPD confidence intervals. The key describes the greater geographic ranges of the species.

| | A. stypheliodes | M. revoluta | M. hamata | M. corymbosa | M. pulvinaris | M. tenuifolia | M. glandulosa | M. sororis | M. deflexa |
|---|---|---|---|---|---|---|---|---|---|
| M. revoluta | **0.98** | | | | | | | | |
| M. hamata | 0 | 0 | | | | | | | |
| M. corymbosa | 0 | 0 | 0 | | | | | | |
| M. pulvinaris | 0 | 0 | 0 | 0 | | | | | |
| M. tenuifolia | 0 | 0 | 0 | 0 | 0 | | | | |
| M. glandulosa | 0 | 0 | **1** | **1** | 0 | 0 | | | |
| M. sororis | 0 | 0 | **1** | **0.16** | 0 | 0 | **0.55** | | |
| M. deflexa | 0 | 0 | 0 | **0.95** | 0 | 0 | **1** | 0 | |
| M. conferta | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Fig. 4.4. Proportions of range overlap between species calculated using distributions estimated by convex-hull polygons.** Comparisons which involve overlapping ranges (i.e. all non-zero values) are indicated in bold; a value of 1 indicates comparisons in which the range of the more narrowly-distributed species is completely embedded within that of the species with the larger range. Species names surrounded by the boxes belong to clade B of the 'core' *Macowania* clade.

## Range overlap analysis

Within *Macowania,* most species pairs exhibit zero range overlap (Fig. 4.4). Of the eight comparisons which do show overlap, only three involve species from the same subclade (*M. revoluta* with *A. stypheliodes* [clade B], and *M. glandulosa* with both *M. sororis* and *M. deflexa*

[clade A]). The overlaps involving *M. glandulosa* may partly be a function of using convex hulls, since both species have crescent-shaped ranges (Fig. 4.1). Moreover, where *M. glandulosa* generally favours sandstone substrates at lower altitudes, the latter two associate with the basaltic substrates of the high scarp (Fig. 4.1), such that the true levels of sympatry between these species pairs may be negligible. The same is not true for *M. revoluta* and *A. styphelioides* which have been observed to co-occur at a number of localities (Fig. 4.1), in wet and dry micro-habitats respectively. A comparison of the proportion of pairwise range overlaps between clades A and B revealed no significant differences between clades ($t = -0.3885$, $df = 19$, $P>0.05$), signalling that range overlap levels are uniformly low in both clades.

## Potential adaptive differentiation

Leaf and capitulum measurements provide a strong degree of morphological discrimination between species from clade B (symbols in shades of green), as indicated on the DFA biplot in Fig. 4.5. Most members of clade B, however, show some degree of overlap with one or more species from clade A (symbols in shades of purple and pink), and all clade A species overlap with at least one other member of their clade. Based on the traits examined, species in clade B thus exhibit greater morphological divergence than those in clade A. Consequently, the Mahalanobis' distances between species pairs within clade A are generally lower than those within clade B, and this difference is significant ($t=4.625$, $df=14$, $P<0.001$) when compared against a randomly generated null. Of the 12 pairwise Mahalanobis' distance comparisons within clade A, all have values of 100 or less, with only three (the comparisons of *M. glandulosa* with *M. deflexa*, *M. pulvinaris* and *M. sororis*) being greater than 30. In contrast, all but one comparison within clade B yield distances greater than 100, and the highest value is 900; clearly morphological divergence in the traits of interest is much higher within this clade.

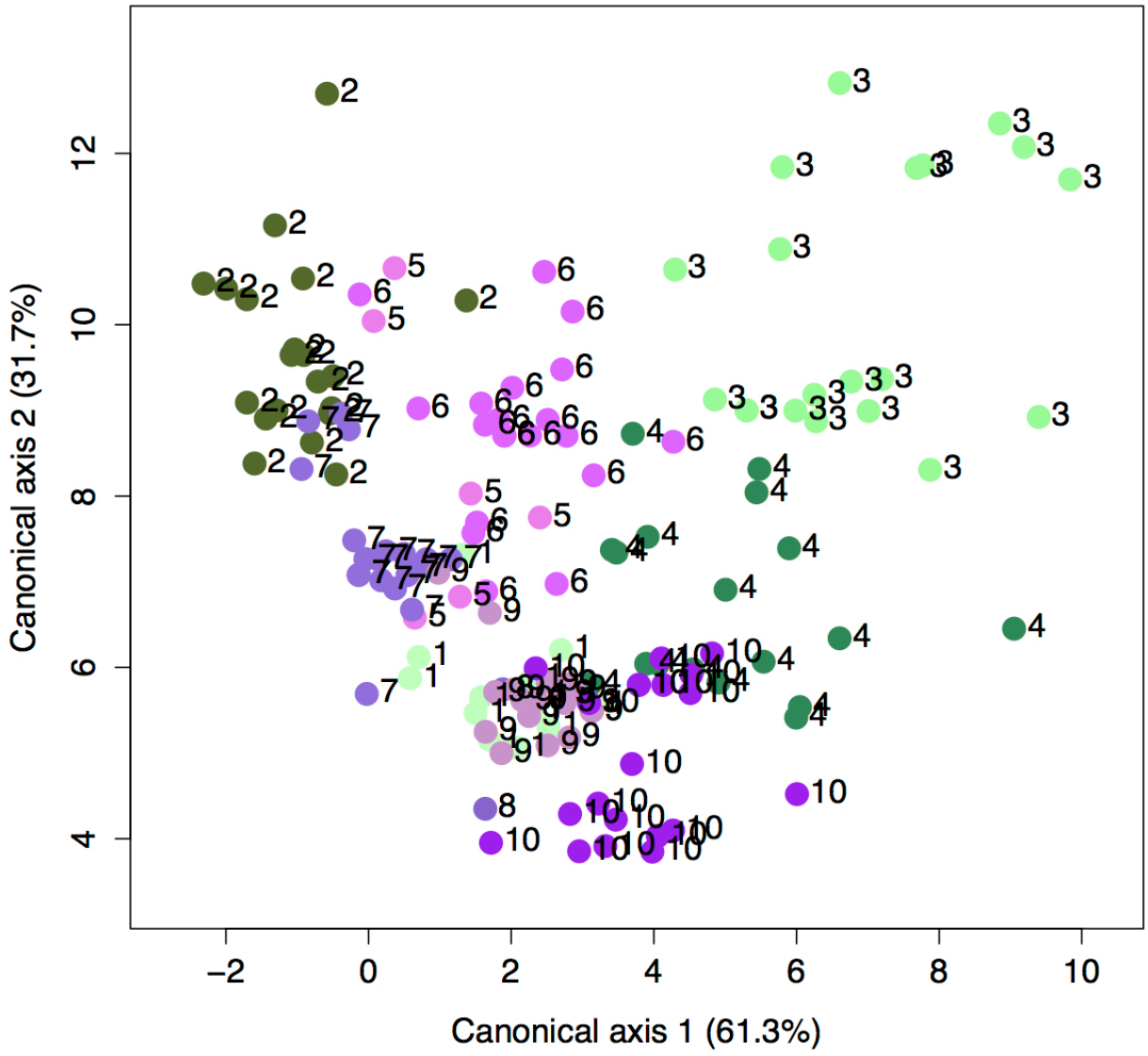



**Fig. 4.5. Discriminant functions analysis biplot of Mahalanobis distances based on capitulum and leaf measurements.** Clade A species are indicated by symbols in shades of purple and pink while clade B species symbols are coloured shades of green. 1=*M. hamata,* 2=*A. styphelioides,* 3=*M. corymbosa,* 4=*M. revoluta,* 5=*M. conferta,* 6=*M. glandulosa,* 7=*M. sororis,* 8=*M. deflexa,* 9=*M. pulvinaris* and 10=*M. tenuifolia.*

# Discussion

This study appears to present the first dated molecular phylogenetic study on a Drakensberg-near-endemic plant lineage as well as the first examination of geographic mode of speciation for the region. Phylogenetic data are fundamental to the study of lineage diversification, not only because they provide a (albeit tentatively) dated record of the successive speciation events underlying present-day species diversity, but also because they identify the bounds and membership of the lineage under study. *Macowania* as a non-monophyletic entity would be inappropriate as a unit for evolutionary study; however, the data in previous chapters reveal that the non-monophyly of *Macowania* is easily rectified by the inclusion of *Arrowsmithia.* The taxonomic implications of a revised generic re-circumscription for *Macowania* is addressed in Chapter Two. Miocene-Pliocene uplift of the Drakensberg appears to have strongly influenced diversification within the *Macowania-Arrowsmithia* clade, because a strong signal of range allopatry points to geographical isolation as a key driver of speciation, and this diversification appears to have occurred post-uplift. Geographical isolation in *Macowania* is thus inferred to be linked to Pliocene uplift and subsequent landscape erosion, these processes providing the necessary impetus for both non-ecological and ecological speciation.

### Pliocene uplift in the Drakensberg

The data are consistent with the scenario of a Drakensberg radiation in *Macowania* in response to recent tectonic uplift, probably in the Early Pliocene. The BEAST analysis dates the origin of the 'core' *Macowania* clade to 6 Ma, coinciding with the Miocene-Pliocene boundary (though this estimate carries appropriately wide error margins: 95%HPD 3.8 – 8.6 Ma). This date is in line with earlier estimates of Drakensberg dispersals and radiations in *Pentaschistis, Disa* and *Morea* (Galley et al. 2007). The bulk of the present-day species diversity is likely to have been generated more recently. The radiation of the two principal clades (clades A and B) is initiated shortly after major

Pliocene uplift of the eastern Escarpment (King & King 1959; Partridge & Maud 1987, 2000; Partridge 1998); the crown ages dated to 4.4 Ma and 4.8 Ma respectively, and thus consistent with a role for post-uplift scarp erosion.

Both subclades exhibit strong signals of range allopatry, suggesting that geographic isolation was important in speciation. Concordant with this idea, *Macowania* lacks specialised features which promote long-distance dispersal. Pronounced allopatry, in which distribution breaks coincide with erosion barriers, supports the idea that scarp erosion played a key role in the diversification of *Macowania.* This is best exemplified by the clade comprising *M. sororis*, *M. deflexa* and *M. conferta*. Species in this lineage consistently associate with high-altitude environments, all three being allopatric and for the most part very narrowly distributed. *Macowania conferta* is restricted to Ngeli Mountain, an isolated peak situated on the coastal plain about 85 km southeast of the main Drakensberg massif. Like Mount Currie, which supports the southeastern-most population of *M. sororis*, Ngeli is a relict fragment of a once-more extensive Drakensberg Escarpment, which has resisted the erosive forces that caused the Escarpment to retreat away from the present-day coastline. As such, the presence of *Macowania* on these peaks is likely also relictual, the disjunct nature of this distribution promoting vicariant divergence. Similarly, the deeply-incised Orange River gorge (Fig. 4.1), the magnitude of its drainage accentuated by the increased westward tilt that Pliocene uplift conferred on the region (Partridge & Maud 1987), might explain the isolated and vicariant presence of *M. pulvinaris* on the opposing side of the river from other *Macowania* members, in western Lesotho and the northern reaches of the Eastern Cape Drakensberg. The presence of *M. tenuifolia* on isolated patches of Afrotemperate habitat in the Mpumalanga, Gauteng and Limpopo provinces might also be attributed to landscape erosion enabling divergence in allopatry. There are several floral elements linking this region with the KwaZulu-Natal Drakensberg (e.g. *Helichrysum subglomeratum, Selago procera*) as well as with the escarpment to the north in

the Zimbabwean highlands (e.g. *H. swynnertonii, Aloe modesta;* [Mucina & Rutherford 2006]), perhaps suggesting the historical connection of this land.

### Speciation of *Macowania* in the Drakensberg

In the context of erosion-mediated vicariance, divergent selection may be critical in powering speciation (ecological speciation) or, somewhat more controversially (Rundell & Price 2009; Schluter 2009), speciation may be powered by neutral processes alone (non-ecological speciation). Weak habitat and morphological differentiation between species within clade A suggest a primary role for isolation in species formation (non-ecological speciation). This is particularly evident for *M. sororis, M. deflexa, M. conferta* and *M. pulvinaris*, which exhibit no range overlap and very little morphological divergence, at least in terms of the traits sampled in this study. Taxonomically, these species are distinguished only by subtle characters of the peduncles and leaf-glands (Hilliard & Burtt 1976).

There is, however, some evidence for ecological divergence within clade A. Although *M. glandulosa* is broadly sympatric with *M. sororis* and *M. deflexa* (the only instances of sympatry in this clade), it shows some evidence of fine-scale ecological differentiation. Where the latter species inhabit scarp edge basalt substrates, *M. glandulosa* favours lower elevations, growing on the sandstone platforms that underlie the basalts (Hilliard & Burtt 1976). Associated with this habitat shift is a morphological transition, *M. glandulosa* being the most morphologically-disparate species in clade A. Indeed, within *Macowania* as a whole, *M. glandulosa* is the only species possessing sunken leaf glands. Interestingly, *M. glandulosa* is itself disjunctly distributed, occupying two separate areas on the northeastern and southeastern arms of the Drakensberg Escarpment (Fig. 4.1). There is some evidence of morphological divergence between the two regions, with the northernmost populations lacking glandular hairs on the leaves (Hilliard & Burtt 1976), indicating

ongoing speciation in allopatry.

In contrast to clade A, adaptive divergence appears to have played a greater role in stimulating speciation in clade B. Though leaf and capitulum measurements capture the stronger morphological differentiation between species within this clade, this is apparent even on the basis of cursory visual examination. For example, striking variation is apparent in leaf morphology, involucral bract coloration (*M. revoluta* and *M. corymbosa* have brown-edged bracts) and capitulum sexuality (in contrast to the typical gynomonoecious condition, *M. revoluta* has dioecious, and *M. corymbosa*, hermaphrodite capitula). These differences are also reflected in the taxonomic history of the group, this clade containing a species which has hitherto been treated as a separate genus (*Arrowsmithia*). Within clade B, morphological differentiation is most pronounced between *M. revoluta* and *A. styphelioides*. This differentiation is almost certainly ecologically motivated, these species being fully sympatric, although they occupy different micro-habitats within their shared range (*M. revoluta* grows in deep sandy soil in bogs, while *A. styphelioides* inhabits rocky slopes).

The other sister-pair within clade B, *M. hamata* and *M. corymbosa*, shows non-overlapping ranges, occurring exclusively on the southern and northern axes of the Drakensberg, respectively. Although these species show strong morphological divergence, their habitats are similar, suggesting a scenario of adaptive divergence in allopatry.

Ecologically-driven flowering time shifts also provide a potentially important mechanism which might power genetic isolation in sympatric species. Herbarium record data reveal little to no flowering time overlap between *M. glandulosa* (October – December) and *M. sororis* (January – July), and only marginal overlap between *M. glandulosa* and *M. deflexa* (December – January), suggesting that differences in phenology might influence the isolation of these species. Conversely,

the data reveal that the flowering times of the sister-pair *A. styphelioides* (May – December) and *M. revoluta* (August – February) overlap; their ecological divergence thus cannot be attributed to differences in flowering time, suggesting that an alternative ecological explanation might explain their divergence in sympatry.

Although small flies have been observed visiting flowers of *Macowania* (N.Bergh, pers. obs.), little is known about the pollination mechanisms and breeding systems of these plants. Several studies (e.g. Arroyo et al. 1982; Elberling & Olesen 1999) find pollinator diversity decreasing with increasing altitude, as well as a dominance of flies at higher altitudes. Galley et al. (2007) suggest that pollinator specificity might play a role in promoting *in situ* speciation, suggesting that taxa with generalist pollination syndromes are unlikely to speciate as readily as those with specialist systems upon entering a new region. The same is suggested for *Euryops* (Devos et al. 2010), as, possibly like *Macowania,* this genus (also well-represented in the Afrotemperate regions) has a typical Asteraceae generalist pollination syndrome.

## Conclusion

A scenario of post-uplift erosion-mediated speciation is sketched in *Macowania*. Although it appears that there are no studies that explore the role of erosion as a stimulus for the diversification of the Drakensberg flora, a major limitation is that the scale of speciation in many Drakensberg clades has been modest, compromising the ability to infer strong patterns. Here, evidence is found in support of a hypothesis of uplift-induced landscape erosion in stimulating both adaptive and non-adaptive speciation. Where species are allopatric, distribution breaks coincide with erosion barriers and species are found to have little morphological differentiation; exemplified in clade A. Alternatively, a signal of adaptive divergence in both sympatric and allopatric species in clade B is apparent.

## General conclusion

The association of relhanioid lineages with both Afrotemperate and arid habitats throughout Africa and the circum-Mediterranean provides a model system for examining dispersal patterns against a backdrop of paleoclimatic changes in the region over the last 20 Ma. The findings of this dissertation support several previous biogeographic hypotheses relating to the dispersal of arid and Afrotemperate pan-African lineages. One novel finding links post-uplift landscape erosion in the Drakensberg to ecological and non-ecological speciation in an Afrotemperate endemic genus. A rigorously-assessed phylogenetic hypothesis for species relationships in the *Relhania* clade provides the skeleton on which to draw these inferences.

As previous studies have found that inadequate taxon sampling could result in phylogenetic error and misplaced taxa (Chase et al. 1993; Greybeal 1998; Zwickl & Hillis 2002), one of the strengths of this study is its near-complete sampling of the *Relhania* clade, with multiple accessions for most species. As such, it presents the first attempt at a robust and well-sampled molecular phylogenetic hypothesis for the entire group. Though molecular markers from both plastid and nuclear regions were sampled, the weakest aspect of the phylogeny is its lack of resolution at some internal nodes of the tree. This is a problem ubiquitous in plant phylogenetic inference, largely attributed to low nucleotide substitution rates especially in mitochondrial and chloroplast DNA (Laroche et al. 1997; Wolfe et al. 1987). Nevertheless, a comprehensively-tested phylogenetic hypothesis reveals that the *Relhania* clade is monophyletic and sister to Gnaphalieae *sensu stricto*, though several lineages within the clade are found to be non-monophyletic. As the addition of morphological data for a subset of taxa did not provide a coherent solution for unresolved lineages, the non-monophyly was addressed through the dissolution of several genera into larger, more comprehensive clades. Most problematic to resolve was the species-rich and morphologically variable Oederinae *sensu stricto,* its diversity being reflected in the fact that it currently houses six genera (*Antithrixia, Comborhiza,*

*Oedera, Oreoleysera, Relhania, Rosenia*).. The generic circumscription of the *Relhania* clade is unlikely to be re-examined with additional data and full species-level sampling by another researcher in the near future. Thus, given the available data, the recognition of fewer, more inclusive genera provides the best taxonomic solution for the foreseeable future.

An Early Miocene origin for relhanioid lineages in southern Africa, most likely in the GCFR in a relatively mesic moisture niche, is inferred by likelihood analysis of ancestral range evolution, a date earlier than the estimated onset of aridification in southwestern Africa (DuPont et al. 2011; Diekmann et al. 2003; Linder et al. 2003; Siesser 1980; Zachos et al. 2001). The ancestor of the *Relhania* clade may thus have originated prior to, or alongside, aridification and possibly may have been refugial in the mountains of the Cape or Karoo Escarpment which, due to their altitude, did not experience the subtropical climate of the lower-lying regions (Linder et al. 1992; Verboom et al. 2009). Evidence is provided in support of northwards migration out of southern Africa following two distinct and perhaps even non-contemporaneous arid and Afrotemperate tracks. While the Namaqualand-Namib domain is not found to be an important southern stepping-stone for arid migrations, the opposite is true for the Afrotemperate track where the Drakensberg is important in the transfer of taxa between the Cape and Afrotemperate highlands (*sensu* Galley et al. 2007). Migrations in the arid lineages occurred from the Late Miocene in *Phagnalon* and then again in the Pliocene in *Leysera*. Though the error bars on these dates appropriately suggest substantial uncertainty, they are nevertheless coincident with arid track migrations estimated in other biogeographic studies in the Late Miocene (Caujape-Castells et al. 2001; del Hoyo et al. 2009; Hernandez-Vera et al. 2013; Thiv et al. 2011) and Pliocene (Bellstedt et al. 2012). Afrotemperate dispersals are subsequent to tectonic uplift and rifting in southern and eastern Africa, the majority of which (with the exception of some species of *Macowania*) occurring from the Pliocene. The Drakensberg dispersal of the ancestor of *M. pinifolia* predates Pliocene uplift but is subsequent to

Miocene uplift, and was probably a colonisation of the sub-alpine zone, possibly via the Karoo Escarpment. The dispersal of *Macowania* into Ethiopia is dated to the Late Miocene, which coincides with estimates for earlier rifting in this region (Chorowics 2005; Flannery & Rosendahl 1990; Wolfenden et al. 2004). The majority of migrations from the GCFR are into the Drakensberg (four instances out of eight), further emphasising its stepping-stone role. The Drakensberg also harbours the only other significant radiation of Oederinae species outside of the GCFR, in *Macowania* (incorporating *Arrowsmithia*). Perhaps significantly, this is the oldest dispersal to the Drakensberg in the *Relhania* clade, and the only one resulting in more than one or two endemic species.

The specific role of landscape erosion associated with Miocene and Pliocene tectonic uplift in stimulating speciation in the Drakensberg has not previously been examined. Tectonic uplift is hypothesised to influence speciation through producing a novel, high-altitude adaptive zone which may serve as an arena for ecological speciation. It might also indirectly promote species divergence in allopatry by stimulating large-scale erosion. A dated phylogenetic hypothesis of the Drakensberg near-endemic genus *Macowania* reveals a crown age coincident with Pliocene tectonic uplift (though the error bars suggest substantial uncertainty). An examination of range overlap patterns and morphological differentiation (a proxy for functional divergence) finds evidence for both adaptive and non-adaptive speciation. Two primary subclades are identified; one (clade A) associating largely with scarp-edge habitats and being mostly allopatrically-distributed with little morphological differentiation, and the other (clade B) occurring in moister habitats, being highly morphologically divergent with two members occurring in sympatry. The greater relative morphological divergence in sympatric species of *Macowania* (clade B) contrasts with the situation in the non-sympatric taxa (clade A), suggesting that simple geographic isolation may have been a stronger force driving speciation (‘neutral speciation’) in clade A. Weak habitat and morphological

differentiation in clade A further suggests a role for non-ecological speciation, particularly evident between a suite of closely-related species distinguished taxonomically only by subtle peduncle and leaf-gland characters *(M. deflexa, M. sororis, M. conferta* and *M. glandulosa*). The limited instances of sympatry in clade A might be attributed to ecological differentiation through substrate adaptation, or possibly phenological isolation as they exhibit no overlap in flowering time. The strong signal of ecological speciation in sympatric members of clade B (*Arrowsmithia* and *M. revoluta*) is further emphasised by their overlapping phenology.

## Future Research

Future research on the phylogeny of the *Relhania* clade might focus on producing a better-resolved phylogenetic hypothesis, especially within the GCFR clade of Oederinae and poorly resolved relationships in *Athrixia*. In addition to the four molecular markers used in this study, sampling more chloroplast markers might produce a better-resolved plastid tree.

The species-rich *Phagnalon* occupies a diversity of moisture niches (Fig. 3.5) as well as both highly restricted and more widespread ranges. More fine-scale teasing apart of their circum-Mediterranean habitats and incorporating these into reconstructions might thus reveal further interesting biogeographic and adaptive patterns. While leaf material was obtained for the Madagascan-endemic *Athrixia debilis,* the DNA extraction proved fruitless. Obtaining nucleotide data for this species and incorporating it into the biogeographic analyses might unveil further insights into the stepping-stone hypothesis and Afrotemperate track, i.e. whether migration to Madagascar commenced directly from the Cape, Drakensberg or East Africa. Some studies indicate that the colonisation of Afrotemperate lineages into Madagascar occurred from southern Africa (Ali et al. 2013), with evidence for direct dispersal from both the Cape and/or the Drakensberg (Eiserhardt et al. 2011; Galley et al. 2007), while others suggest dispersal from East Africa (Gehrke & Linder 2009).

*Athrixia debilis* is morphologically closely allied with *A. rosmarinifolia* (Kroner 1980), which has a broad geographic range spanning the highlands of Zimbabwe to Ethiopia. It might then be assumed that dispersal to Madagascar probably commenced from East Africa. A more detailed examination of habitat variables might provide more compelling evidence for a phylogenetic niche conservatism hypothesis. For instance, reconstructions of other variables such as altitude or indicators of aridity tolerance (including, for example, precipitation of the driest month/quarter). The provision of a satisfactory null hypothesis, perhaps based on permutation of phylogenetic tree tips, might provide a statistical sample against which to evaluate habitat shifts and their association with range expansions.

Studies on the role of uplift and erosion in the Drakensberg on the radiation of plant lineages are limited by the scale of speciation in many Drakensberg clades. Nevertheless, this idea could be explored in the light of phylogenetic hypotheses of the larger endemic lineages, such as *Helichrysum* and *Senecio* (Asteraceae; 29 and 22 species, respectively); *Erica* (Ericaceae; 12 species), *Delosperma* (Mesembryanthemaceae; 12 species), *Glumicalyx* (Scrophulariaceae; 6 species), *Rhodohypoxis* (Hypoxidaceae; 6 species) and *Huttonaea* (Orchidaceae; 6 species) whose radiations, like that of *Macowania*, are hypothesised to have followed major uplift at the Miocene-Pliocene boundary (e.g. Galley et al. 2007). These findings may indicate general patterns of diversification applicable to other tectonically-influenced systems, including the high Andes, and suggest that post-uplift habitat production by erosive processes might be as much of a driver of speciation as the initial uplift itself.

# References


Adamson, R.S. (1958). The Cape as an ancient African flora. *The Advancement of Science*, 58: 1–10.

Agusti, J., Sanz de Siria, A. & Garces, M. (2003) Explaining the end of the hominoid experiment in Europe. *Journal of Human Evolution*, *45:* 145–153.

Akaike, H. (1973). Information theory and an extension of the maximum-likelihood principle. In Second Int. Symp. Inform. Theory, Tsahkadzor, Armenia, USSR (ed. B. N. Petrov & F. Csaki), pp. 267–281. Budapest: Akademiai Kiado.

Akaike, H. (1994). A new look at the statistical model identification. *Automatic Control, IEEE Transactions*, 19:716–723.

Alfaro, M. E., & Holder, M. T. (2006). The Posterior and the Prior in Bayesian Phylogenetics. *Annual Review of Ecology, Evolution and Systematics:* 19–42.

Ali, S. S., Pfosser, M., Wetschnig, W., Martínez-Azorín, M., Crespo, M. B., & Yu, Y. (2013). Out of Africa: Miocene dispersal, vicariance and extinction within Hyacinthaceae subfam. Urgineoideae. *Journal of Integrative Plant Biology:* 1–33.

Allen, J. F., & Raven, J. A. (1996). Free-radical-induced mutation vs redox regulation: costs and benefits of genes in organelles. *Journal of Molecular Evolution*, *42*(5): 482–92.

Anderberg, A. A. (1989). Phylogeny and reclassification of the tribe Inuleae (Asteraceae). *Canadian Journal of Botany*, *67*(8): 2277–2296.

Anderberg, A. A. (1991). Taxonomy and phylogeny of the tribe Gnaphalieae (Asteraceae). *Opera Botanica*, *104:* 1–195.

Anderberg, A. A., & Bremer, K. (1991). Parsimony analysis and cladistic reclassification of the Relhania generic group (Asteraceae-Gnaphalieae). *Annals of the Missouri Botanical Garden:* 1061–1072.

Anderberg, A. A., & Källersjö, M. (1988). The tribal position of Oedera L.(Compositae). *Botanical Journal of the Linnean Society*, *96*(4): 323–332.

Alvarez, I., & Wendel, J. F. (2003). Ribosomal ITS sequences and plant phylogenetic inference.

*Molecular Phylogenetics and Evolution*, *29:* 417–434.

Arroyo, M.T.K., Primack, R. & Armesto, J. (1982). Community studies in pollination ecology in the high temperate Andes of Central Chile. I. Pollination mechanisms and attitudinal variation. *American Journal of Botany*, 69**:** 82–97.

Axelrod, D.I. (1975) Evolution and biogeography of the Madrean-Tethyan sclerophyllous vegetation. *Annals of the Missouri Botanical Garden*, 62: 280–334.

Axelrod, D. I. & Raven, P. H. (1978). Late Cretaceous and Tertiary vegetation history of Africa. In Biogeography and ecology of Southern Africa (ed. M. J. A. Werger), pp. 77–130. The Hague, The Netherlands: Junk.

Backlund, A., & Bremer, K. (1998). To be or not to be. Principles of classification and monotypic plant families. *Taxon*, *47*(2): 391–400.

Baldwin, B. G., & Markos, S. (1998). Phylogenetic Utility of the External Transcribed Spacer (ETS) of 18S–26S rDNA: Congruence of ETS and ITS Trees of Calycadenia (Compositae). *Molecular Phylogenetics and Evolution*, *10*(3): 449–463.

Baldwin, B. G., Sanderson, M. J., Porter, J. M., Wojciechowski, M. F., Campbell, C. S., & Donoghue, M. J. (1995). The its region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden*, *82*(2): 247–277.

Balinsky, B.I. (1962) Patterns of animal distribution of the African continent. Annals of the Cape Provincial Museum, 2: 299–310.

Barreda, V. D., L. Palazzesi, M. C. Tellería, L. Katinas, and J. V. Crisci. (2010). Fossil pollen indicates an explosive radiation of basal Asteracean lineages and allied families during Oligocene and Miocene times in the Southern Hemisphere. *Review of Palaeobotany and Palynology,* 160 (3- 4): 102–110.

Barreda, V. D., L. Palazzesi, M. C. Telleria, L. Katinas, J. V. Crisci, K. Bremer, M. G. Passalia, R. Corsolini, R. Rodriguez Brizuela, and F. Bechis. (2010b). Eocene Patagonia fossils of the daisy family. *Science,* 329: 1621.

Bayer, R. J., Christopher F. P., and S. A. Kelchner. (2000). Phylogeny of South African Gnaphalieae (Asteraceae) based on two noncoding chloroplast sequences. *American Journal of Botany* 87.2: 259–272.

Bayer, R. J., D. G. Greber and N. H. Bagnall. (2002). Phylogeny of Australian Gnaphalieae (Asteraceae) based on chloroplast and nuclear sequences, the trnL intron, trnL /trnF intergenic spacer, matK , and ETS. *Systematic Botany, 27:* 801–814.

Bayer, M. B., Breitweiser, I., Ward, J., Puttock, C.F. (2007). Tribe Gnaphalieae. In: Kubitzki, K. (Ed.), The Families and Genera of Flowering Plants, Flowering Plants, Eudicots, Asterales, vol. VIII. Springer, Berlin, pp. 246–283.

Bellstedt, D. U., Galley, C., Pirie, M. D., & Linder, H. P. (2012). The Migration of the Palaeotropical Arid Flora: Zygophylloideae as an Example. *Systematic Botany*, *37*(4): 951–959.

Bergh, N. G., & Peter Linder, H. (2009). Cape diversification and repeated out-of-southern-Africa dispersal in paper daisies (Asteraceae–Gnaphalieae). *Molecular Phylogenetics and Evolution*, *51*(1): 5–18.

Bergh, N. G., Trisos, C. H., & Verboom, G. A. (2011). Phylogeny of the" Ifloga clade" (Asteraceae, Gnaphalieae), a lineage occurring disjointly in the Northern and Southern Hemisphere, and inclusion of Trichogyne in synonymy with Ifloga. *Taxon*, *60*(4): 1065–1075.

Biondi, M. & D'Alessandro, P. (2008) Taxonomical revision of the Longitarsus capensis species-group: an example of Medi- terranean-southern African disjunct distributions (Coleoptera: Chrysomelidae). *European Journal of Entomology*, 105: 719–736.

Blöch, C., Weiss-Schneeweiss, H., Schneeweiss, G. M., Barfuss, M. H. J., Rebernig, C. a, Villaseñor, J. L., & Stuessy, T. F. (2009). Molecular phylogenetic analyses of nuclear and plastid DNA sequences support dysploid and polyploid chromosome number changes and reticulate evolution in the diversification of Melampodium (Millerieae, Asteraceae). *Molecular Phylogenetics and Evolution*, *53*(1): 220–33.

Bobe, R. (2006). The evolution of arid ecosystems in eastern Africa. *Journal of Arid Environments*, *66*(3): 564–584.

Bologna, M.A., Di Giulio, A. & Pitzalis, M. (2008) Systematics and biogeography of the genus Actenodia (Coleoptera: Meloidae: Mylabrini). *Systematic Entomology*, 33: 319–360

Born, J., Linder, H. P., & Desmet, P. (2007). The Greater Cape Floristic Region. *Journal of Biogeography*, *34:* 147–162.

Bremer, K. (1976). The genus Relhania (Compositae). *Opera Botanica*, (40).

Bremer, K. (1976b). The genus Rosenia (Compositae). *Botaniska Notiser*, *129*(2): 97–111.

Bremer, K. (1978). The genus Leysera (Compositae). *Botaniska Notiser, 131*(3): 369-383.

Bremer, K., Friis, E. & Bremer, B. (2004). Molecular phylogenetic dating of asterid flowering plants shows early Cretaceous diversification. *Systematic Biology*, *53*(3): 496-505.

Bryson, R. W., Murphy, R. W., Lathrop, A., & Lazcano-Villareal, D. (2011). Evolutionary drivers of phylogeographical diversity in the highlands of Mexico: a case study of the Crotalus triseriatus species group of montane rattlesnakes. *Journal of Biogeography*, 38:697–710.

Bryson, R. W., García-Vázquez, U. O., & Riddle, B. R. (2012). Diversification in the Mexican horned lizard Phrynosoma orbiculare across a dynamic landscape. *Molecular Phylogenetics and Evolution*, 62: 87–96.

Bryson, R. W., García-Vázquez, U. O., & Riddle, B. R. (2012) Relative roles of Neogene vicariance and Quaternary climate change on the historical diversification of bunchgrass lizards (Sceloporus scalaris group) in Mexico. *Molecular Phylogenetics and Evolution*, 62:447–57.

Burgman, M. A., & Fox, J. C. (2003). Bias in species range estimates from minimum convex polygons: implications for conservation and options for improved planning. *Animal Conservation*, 6:19–28.

Burke, K. & Gunnell, Y. (2008). The African erosion surface: A continental-scale synthesis of geomorphology, tectonics, and environmental change over the past 180 million years, Geological Society of America Memoir 201.

Burnham, R. J., & Graham, A. (1999). The history of Neotropical vegetation: new developments and status. *Annals of the Missouri Botanical Gardens*, 86:546–589.

Calenge, C. (2006). The package “adehabitat” for the R software: A tool for the analysis of space

and habitat use by animals. *Ecological Modelling*, 197:516–519.

Calviño CI, Tilney PM, van Wyk B-E, Downie SR. (2006). A molecular phylogenetic study of the southern African Apiaceae. *American Journal of Botany,* 93:1828–1847.

Carbutt, C., & Edwards, T. J. (2004). The flora of the Drakensberg Alpine Centre. *Edinburgh Journal of Botany*, *60*(03): 581–607.

Carbutt, C., & Edwards, T. J. (2006). The endemic and near-endemic angiosperms of the Drakensberg Alpine Centre. *South African Journal of Botany*, *72*(1): 105–132.

Carlson, S. E., Linder, H. P., & Donoghue, M. J. (2012). The historical biogeography of Scabiosa (Dipsacaceae): implications for Old World plant disjunctions. *Journal of Biogeography*, *39*(6): 1086–1100.

Caujapé-Castells, J., Jansen, R.K., Membrives, N., Pedrola-Monfort, J., Montserrat, J.M., Ardanuy, A., (2001). Historical biogeograpy of Androcymbium Willd. (Colchicaceae) in Africa: evidence from cpDNA RFPLs. *Botanical Journal of the Linnean Society,* 136: 379–392.

Cerling, T. E., Harris, J. M., Macfadden, B. J., Leakey, M. G., Quadek, J., Eisenmann, V., & Ehleringer, J. R. (1997). Global vegetation change through the Miocene/Pliocene boundary. *Nature*, *389:* 153–158.

Eiserhardt, W. L., Rohwer, J. G., Russell, S. J., Yesilyurt, J. C., & Schneider, H. (2011). Evidence for radiations of cheilanthoid ferns in the Greater Cape Floristic Region. *Taxon*, *60*(5): 1269–1283.

Chorowicz, J. (2005) The East African rift system. Journal of African Earth Sciences, 43, 379–410.

Coetzee, J. A. (1978). Late Cainozoic palaeoenvironments of southern Africa. In Antarctic Glacial History and World Palaeoenvironments, edited by E. M. van Zinderen-Bakker. Rotterdam: Balkema.

Coetzee, J.A., (1983). Intimations on the Tertiary vegetation of southern Africa. *Bothalia* 14: 345–354.

Coleman, M., Liston, A., Kadereit, J. W., & Abbott, R. J. (2003). Repeat intercontinental dispersal

and Pleistocene speciation in disjunct Mediterranean and desert Senecio (Asteraceae). *American Journal of Botany*, *90*(10): 1446–54.

Compton, R. (1931). Alatoseta tenuis. *Transactions of the Royal Society of South Africa*, (19), 314.

Cowling, R.M., Gibbs Russell, G.E., Hoffmann, M.T. & Hilton-Taylor, C. (1989). Patterns of plant species diversity in southern Africa. In: Huntley, B.J. (ed.), *Biotic diversity in southern Africa: concepts and conservation,* pp. 19 – 50. Oxford Uni. Press, Cape Town.

Cowling, R.M., Holmes, P.M. & Rebelo, A.G. (1992). Plant diversity and endemism. In: Cowling, R.M. (ed.), *The ecology of fynbos: nutrients, fire and diversity,* pp. 62 – 112. Oxford Uni. Press, Cape Town.

Cowling, R.M., Rundel, P.W., Lamont, B.B., Arroyo, M.K. & Arianoutsou, M. (1996) Plant diversity in mediterranean-climate regions. *TREE Reviews,* 11(9): 362–366.

Crisp, M. D., Arroyo, M. T. K., Cook, L. G., Gandolfo, M. A., Jordan, G. J., Mcglone, M. S., … Linder, H. P. (2009). Phylogenetic biome conservatism on a global scale. *Nature*, *458*(April): 754–756.

Davies, T.A., Hay, W.W., Southam, J.R., & Worsley, T.R. (1977). Estimates of Cenozoic oceanic sedimentation rates. *Science,* 197**:** 53–55.

del Hoyo, A., García-Marín, J. L., & Pedrola-Monfort, J. (2009). Temporal and spatial diversification of the African disjunct genus Androcymbium (Colchicaceae). *Molecular Phylogenetics and Evolution*, *53*(3): 848–61.

deMenocal, P. B. (1995). Plio-Pleistocene African climate. *Science (New York, N.Y.)*, *270*(5233): 53–59.

deMenocal, P. B. (2004). African climate change and faunal evolution during the Pliocene–Pleistocene. *Earth and Planetary Science Letter*s, 220 (1-2): 3–24.

Devos, N., Barker, N.P., Nordenstam, B & Mucina, L. (2010). A multi-locus phylogeny of *Euryops* (Asteraceae, Senecioneae) augments support for the “Cape to Cairo” hypothesis of floral migrations in Africa. *TAXON,* 59(1): 57–67.

Dieckmann, B., Falker, M., Kuhn, G., (2003). Environmental history of the southeastern South Atlantic since the middle Miocene: evidence from the sedimentological records of ODP sites 1088–1092. *Sedimentology,* 50: 511–529.

Doyle, J., & Doyle, J. L. (1987). Genomic plant DNA preparation from fresh tissue-CTAB method. *Phytochemical Bulletin*, 19.

Doyle, J.A.,Donoghue, M. J., and Zimmer, E.A. (1994). Integration of morphological and ribosomal RNA data on the origin of the angiosperms. *Annals of the Missouri Botanical Garden.* 81: 419-450.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology,* 4: 699–710. Dumolin-Lapègue.

Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, *7*(1): 214.

Dupont, L.M., (2006). Late Pliocene vegetation and climate in Namibia (southern Africa) derived from palynology of ODP Site 1082. *Geochemistry. Geophyicss. Geosystems.* 7.

Dupont, L.M.,Donner, B., Vidal, L., Pérez, E.M.,Wefer, G. (2005). Linking desert evolution and coastal upwelling: Pliocene climate change in Namibia. *Geology, 3*3: 461–464.

Dupont, L. M., H. P. Linder, F. Rommerskirchen, and E. Schefub. (2011). Climate-driven rampant speciation of the Cape flora. *Journal of Biogeography*, 38: 1059–1068.

Edwards, A. W. (1992). Likelihood, expanded ed. *Baltimore: Johns Hopkins*.

Elberling, H. & Olesen, J.M. (1999). The structure of a high latitude plant-flower visitor system: the dominance of flies. *Ecography*, 22**:**314-323.

Eldenäs, P.K., Linder, H.P., (2000). Congruence and complementarity of morphological and trnL-trnF sequence data and the phylogeny of the African Restionaceae. *Systematic Botany,* 25: 692 – 707.

Felsenstein, J. (1978). Cases in which parsimony or compatibility methods will be positively misleading. *Systematic Zoology***:** 401–410.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap**.**

*Evolution*, 39:783–791.

Fitzpatrick, B. M., & Turelli, M. (2006). The geography of mammalian speciation: mixed signals from phylogenies and range maps. *Evolution*, 60:601–15.

Flannery, J.W., Rosendahl, B.R. (1990). The seismic stratigraphy of Lake Malawi, Africa: implications for interpreting geological processes in lacustrine rifts. *Journal of African Earth Sciences,* 10(3): 519–548.

Forest, F. (2009). Calibrating the Tree of Life: Fossils, molecules and evolutionary timescales. *Annals of Botany* 104 (5): 789–94.

Forest, F., Nänni, I., Chase, M.W., Crane, P.R., Hawkins, J.A., (2007). Diversification of a large genus in a continental biodiversity hotspot: temporal and spatial origin of Muraltia (Polygalaceae) in the Cape of South Africa. *Molecular Phylogenetics and Evolution*, 43: 60–74.

Fortelius, M., Eronen, J., Liu, L., Pushkina, D., Tesakov, A., Vislobokova, I.&Zhang, Z. (2006) Late Miocene and Pliocene large land mammals and climatic changes in Eurasia. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 238: 219–227.

Funk, V.A., Bayer, R.J., Keeley, S., Chan, R., Watson, L., Gemeinholzer, B., Schilling, E., Panero, J.L., Baldwin, B.G., Garcia-Jacas, N., Susanna, A., Jansen, R.K., (2005). Everywhere but Antarctica: using a supertree to understand the diversity and distribution of the Compositae. *Biologiske Skrifter,* 55: 343–374.

Galbany-Casals, M., Garcia-Jacas, N., Susanna, A., Sáez, L., & Benedí, C. (2004). Phylogenetic relationships in the Mediterranean Helichrysum (Asteraceae, Gnaphalieae) based on nuclear rDNA ITS sequence data. *Australian Systematic Botany*, *17*(3): 241–253.

Galbany□Casals, M., Garcia□Jacas, N., Sáez, L., Benedí, C., & Susanna, A. (2009). Phylogeny, Biogeography, and Character Evolution in Mediterranean, Asiatic, and Macaronesian Helichrysum (Asteraceae, Gnaphalieae) Inferred from Nuclear Phylogenetic Analyses. *International Journal of Plant Sciences*, *170*(3): 365–380.

Galbany-Casals, M., Andrés-Sánchez, S., Garcia-Jacas,N., Susanna,A., Rico, E. & Montserrat

Martínez-Ortega, M. (2010). How many of Cassini anagrams should there be? Molecular systematics and phylogenetic relationships in the *Filago* group (Asteraceae, Gnaphalieae), with special focus on the genus *Filago*. *Taxon*, 59:1671–1689.

Galley, C., & Linder, H. P. (2006). Geographical affinities of the Cape flora, South Africa. *Journal of Biogeography*, *33*(2): 236–250.

Galley, C., Bytebier, B., Bellstedt, D. U., & Peter Linder, H. (2007). The Cape element in the Afrotemperate flora: from Cape to Cairo?. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1609): 535–543.

Gavel N. J. & Jarrett R. L. (1991). A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. *Plant Molecular Biology Reporter*, 9: 262–6.

Gehrke, B., & Linder, H. P. (2009). The scramble for Africa: pan-temperate elements on the African high mountains. *Proceedings. Biological sciences / The Royal Society*, *276*(1667): 2657–65.

Gielly, L., & Taberlet, P. (1994). The use of chloroplast DNA to resolve plant phylogenies: noncoding versus rbcL sequences. *Molecular Biology and Evolution*, *11*(5): 769–777.

Gilchrist, A.R. & Summerfield, M.A. (1994). Tectonic models of passive margin evolution and their implications for theories of long-term landscape development. In *Process models and theoretical geomorphology.* Edited by Kirkby M.J. Chichester, UK: John Wiley and Sons :55 84.

Gilks, W. R., Richardson, S., & Spiegelhalter, D. J. (1996). Introducing markov chain monte carlo. *Markov chain Monte Carlo in practice*, 1–19.

Givnish, T. J., & Sytsma, K. J. (1997). Consistency, characters, and the likelihood of correct phylogenetic inference. *Molecular Phylogenetics and Evolution*, *7*(3): 320–30.

Goldblatt, P. (1978) Analysis of the flora of southern Africa: its characteristics, relationships, and origins. Annals of the Missouri Botanical Garden, 65: 369–436.

Goldblatt, P., & Manning, J. C. (2002). Plant diversity of the Cape region of South Africa. *Annals of the Missouri Botanical Garden*, *89*(2): 281–302.

Graham, A. (1996). A contribution to the geologic history of the Compositae. In Compositae: systematics. Proceedings of the International Compositae Conference, Kew, 1994. edited by D. J. N. Hind and H. J. Beenje., Royal Botanic Gardens, Kew, UK.

Graur, D., & Martin, W. (2004). Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends in Genetics*, 20:80–6.

Graybeal, A. (1998). Is it better to add taxa or characters to a difficult phylogenetic problem? *Systematic Biology,* 47: 9–17.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series*,41: 95–98.

Hennig, W. (1950). Grundzuege einer Theorie der phylogenetischen Systematik.

Hennig, W. (1965). Phylogenetic systematics. *Annual Review of Entomology*, (10): 97–116.

Hernández-Vera, G., Caldara, R., Toševski, I., & Emerson, B. C. (2013). Molecular phylogenetic analysis of archival tissue reveals the origin of a disjunct southern African-Palaearctic weevil radiation. (M. Carine, Ed.). *Journal of Biogeography*, *40*(7): 1348–1359.

Hedges, S. B., & Sibley, C. G. (1994). Molecules vs. morphology in avian evolution: the case of the "pelecaniform" birds. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(21): 9861–5.

Hilliard, O. M. (1978). The geographical distribution of Compositae native to Natal: V. *Notes from the Royal Botanic Gardens of Edinburgh*, *34:* 253-86.

Hilliard, O. M., & Burtt, B. (1976). Macowania. *Notes from the Royal Botanical Gardens*, 34:260–279.

Hilliard, O. M., & Burtt, B. L. (1985). Notes on some plants of southern Africa chiefly from Natal: 11. *Notes from the Royal Botanic Gardens of Edinburgh*, *42*(2): 227–260.

Hilliard, O. M., & Burtt, B. L. (1987). The botany of the southern Natal Drakensberg. *Annals of Kirstenbosch Botanic Gardens*, *15*.

Hillis, D. M., & Bull, J. J. (2009). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, *42*(2): 182–192.

Himmelreich, S., Källersjo, M., Eldenäs, P.K., Oberprieler, C., (2008). Phylogeny of Southern Hemisphere Compositae-Anthemideae based on nrDNA ITS and cpDNA ndhF sequence information. *Plant Systematics and Evolution.* 272: 131–153.

Hoffmann, V. (2012). Plants as biotic indicators of Neogene palaeoenvironmental evolution in the Cape Floristic Region. Masters dissertation. Botany Department, University of Cape Town, Cape Town.

Holland, P. G. (1978). An evolutionary biogeography of the genus Aloe. *Journal of Biogeography,* 5: 213–226.

Hughes, C., & Eastwood, R. (2006). Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. *Proceedings of the National Acadademy of Sciences USA,* 103:10334–9.

Huelsenbeck, J.P. (1991) When are fossils better than extant taxa in phylogenetic analysis? *Systematic Zoology*, 40: 458–469.

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*(8): 754–755.

Hughes, C., & Eastwood, R. (2006). Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(27): 10334–9.

Ivanov, D., Ashraf, A.R., Mosbrugger, V. & Palamarev, E. (2002) Palynological evidence for Miocene climate change in the Forecarpathian Basin (Central Paratethys, NW Bulgaria). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 178:19–37.

Jürgens, N. 1997. Floristic biodiversity and history of African arid regions. *Biodiversity Conservation,* 6: 495-514.

Kearney, M. (2002). Fragmentary taxa, missing data, and ambiguity: mistaken assumptions and conclusions. *Systematic Biology*, 51: 369–381.

Killick, D.J.B. (1978). The Afro-alpine Region. In: Davis, S.D., Heywood, V.H. & Hamilton, A.C. (eds.), *Centres of plant diversity. A guide and strategy for their conservation.* Volume 1, pp.

257–259. IUCN Publ., Cambridge.

Killick, D.J.B. (1994). Drakensberg Alpine Region – Lesotho and South Africa. In *Centres of Plant Diversity.* Edited by Davis S. D. & Heywood V. H. Oxford: Oxford University Press:257–260.

King, L. C. (1978). The geomorphology of central and southern Africa. In *Biogeography and ecology of Southern Africa* (ed. M. J. A. Werger). The Hague, The Netherlands: Dr.W. Junk bv Publishers.

King, L. C., & King, L. A. (1959). A reappraisal of the Natal monocline. *South African Geographical Journal*, 41:15-30.

Klaassen, E. S., & Bergh, N. G. (2012). Inclusion of the Cape genus *Anisothrix* in the Namibian-centred genus *Pentatrichia* (Asteraceae, Gnaphalieae) based on a molecular phylogenetic analysis. *South African Journal of Botany*, *78:* 2–11.

Klaassen, E. S., Kwembeya, E. G., & Maass, E. (2009). A taxonomic revision of the genus Pentatrichia. *South African Journal of Botany*, *75:* 153–164.

Klak, C., Reeves, G. & Hedderson, T. (2004). Unmatched tempo of evolution in southern African semi-desert ice plants. *Nature,* 427: 63–65.

Kozak, K.H. & Wiens, J.J. (2006) Does niche conservatism promote speciation? A case study in North American salamanders. *Evolution*, 60: 2604–2621.

Kroner, G. (1980). Systematische Studien im Umkreis von Athrixia Ker-Gawler (Asteraceae). *Mitt Bot Staatssamml München*, *16:* 1–267.

Lageat, Y, & Robb, J.L. (1984). The relationships between structural landforms, erosion surfaces, and the geology of the Archean granite basement in the Barberton region, Eastern Transvaal. *Transactions of the Geological Society of South Africa*, 87:141–159.

Lande, R. (1976). Natural selection and random genetic drift in phenotypic evolution. *Evolution* : 314–334.

Laroche, J., Li, P., Maggia, L., & Bousquet, J. (1997). Molecular evolution of angiosperm mitochondrial introns and exons. *Proceedings of the National Academy of Sciences*, *94*(11): 5722–5727.

Lavin , M. , B. P. Schrire , G. Lewis , R. T. Pennington , A. Delgado- Salinas , M. Thulin , C. E. Hughes, et al. (2004). Metacommunity process rather than continental tectonic history better explains geographically structured phylogenies in legumes. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 359

Levin, N. E., Quade, J., Simpson, S. W., Semaw, S., & Rogers, M. (2004). Isotopic evidence for Plio-Pleistocene environmental change at Gona, Ethiopia. *Earth and Planetary Science Letters,* 219**:** 93–110.

Linder, H.P. (1983). The historical phytogeography of the Disinae (Orchidaceae). *Bothalia*,14: 565–70.

Linder, H. P. (1990). On the relationship between the vegetation and floras of the Afromontane and the Cape regions of Africa. In *Proceedings of the Twelfth Plenary Meeting of AETFAT, Hamburg, September 4-10.* (Vol. 23, pp. 777–790).

Linder, H. P. (1994). Afrotemperate phytogeography: implications of cladistic biogeographical analysis. In *Proc. XIIIth Plenary Meeting AETFAT*, Malawi, vol. 2 (ed. A. C. Chikuni), pp. 913–930. Malawi, Africa: National Herbarium and Botanic Gardens.

Linder, H.P. (2001). On areas of endemism, with an example from the African Restionaceae. *Systematic Biology.* 50: 892–912.

Linder, H.P. (2003). The radiation of the Cape Flora, southern Africa. *Biological Reviews* (Cambridge) 78: 597–638.

Linder, C.R., Goertzen, L.R., Vanden Heuvel, B., Francisco-Ortega,J., Janzen, R.K., (2000). The complete external transcribed spacer of 18S-26S rDNA: amplification and phylogenetic utility at low taxonomic levels in Asteraceae and closely allied families. *Molecular Phylogenetics and Evolution.* 14: 285–303.

Linder, H.P., Meadows, M.E. & Cowling, R.M. (1992). History of the Cape flora. In: Cowling, R.M. (ed.), *The ecology of fynbos: fire, nutrients and diversity,* pp. 113-134. Oxford Uni. Press, Cape Town.

Linder, H.P. Eldenas, P. & Briggs, B. (2003). Contrasting patterns of African and Australian

Restionaceae. *Evolution,* 57: 2688–2702.

Linder, H.P., Hardy, C.R. & Rutschmann F. (2005). Taxon sampling effects in molecular dating: an example from the African restionaceae. *Molecular Phylogenetics and Evolution.* 35: 569-582.

Luteyn, J. L., Churchill, S. P., Griffin III, D., Gradstein, S. R., Sipman, H. J. M., & Gavilanes, A. (1999). Páramos. A checklist of plant diversity, geographical distribution, and botanical literature. New York Botanical Garden.

Mahalanobis, P. C. (1936). On the generalized distance in statistics. *Proceedings of the National Institute of Sciences (Calcutta)*, 2: 49–55.

Markos, S., & Baldwin, B. G. (2001). Higher-level relationships and major lineages of Lessingia (Compositae, Astereae) based on nuclear rDNA internal and external transcribed spacer (ITS and ETS) sequences. *Systematic Botany*, *26*(1): 168–183.

Marlow, J. R., C. B. Lange, G.Wefer, and A. Rosell-Mele. (2000). Upwelling intensification as part of the Pliocene-Pleistocene climate transition. *Science,* 290: 2288–2291.

Martinez-Millan, M. (2010). Fossil record and age of the Asteridae. *Botanical Reviews,* 76: 83–135

Mayr, E., & Bock, W. J. (2002). Classifications and other ordering systems, *40*(August): 169–194.

Mcguire, A. F., & Kron, A. K. (2005). Phylogenetic Relationships of European and African Ericas. *International Journal of Plant Sciences*, *166*(2): 311–318.

McCarthy, T., and B. Rubidge. (2005). The Story of Earth and Life. A southern African Perspective on a 4.6-Billion-Year Journey. Cape Town: Struik Publishers.

Mckinnon, J. S., Mori, S., Blackman, B. K., David, L., Kingsley, D. M., Jamieson, L., Chou, J., et al. (2004). Evidence for ecology's role in speciation. *Nature,* 429**:** 294–298.

Mitsui, Y., Chen, S.-T., Zhou, Z.-K., Peng, C.-I., Deng, Y.-F., & Setoguchi, H. (2008). Phylogeny and biogeography of the genus Ainsliaea (Asteraceae) in the Sino-Japanese region based on nuclear rDNA and plastid DNA sequence data. *Annals of Botany*, *101*(1): 111–24.

Mittermeier, R.A., Meyers, N., Robles, G.P. & Mittermeier, G.C. (2000). *Hotspots: earth's biologically richest and most endangered terrestrial ecoregions:* CEMEX Conservation

International, Mexico City.

Moore, A. E. (1999). A reappraisal of epeirogenic flexure axes in southern Africa. *South African Journal of Geology,* 102 (4): 363–376.

Moore, M.J., Francisco-Ortega, J., Santos-Guerra, A., Jansen, R.K., (2002). Chloroplast DNA evidence for the roles of island colonization and extinction in Tolpis (Asteraceae: Lactuceae). *American Journal of Botany,* 89: 518–526.

Moore, A., & Blenkinsop, T. (2006). Scarp retreat versus pinned drainage divide in the formation of the Drakensberg escarpment, southern Africa. *South African Journal of Geology*, *109:* 599–610.

Moore, A. E., Cotterill, F. P. D. W., Main, M. P. L., & Williams, H. B. (2007). The Zambezi River. In *Large Rivers: Geomorphology and Management.* Edited by Gupta A. England: John Wiley & sons:311–332.

Montes-Moreno, N., Sáez, L., Benedí, C., Susanna, A., & Garcia-Jacas, N. (2010). Generic delineation, phylogeny and subtribal affinities of Phagnalon and Aliella (Compositae, Gnaphalieae) based on nuclear and chloroplast sequences. *Taxon*, *59*(6): 1654–1670.

Montes-Moreno, N., Garcia-Jacas, N., Benedí, C., & Sáez, L. (2013). Evaluation of the taxonomic status of the genus Aliella (Compositae, Gnaphalieae): a recircumscription of the genus Phagnalon. *Phytotaxa*, *148*(1): 1–31.

Morgan, D. A. (2003). nrDNA external transcribed spacer (ETS) sequence data, reticulate evolution, and the systematics of Machaeranthera (Asteraceae). *Systematic Botany*, *28*(1): 179–190.

Mucina, L. & Rutherford, M.C. (2006). The vegetation of South Africa, Lesotho and Swaziland. In *Strelitzia 19.* Edited by Mucina L & Rutherford M.C. Pretoria: South African National Biodiversity Institute: 585-614.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature.* 403: 853–858.

Novacek, M. J. (1992).Fossils, topologies, missing data, and the higher level phylogeny of eutherian mammals, *Systematic Biology,* 41: 58–73.

Nylander, J. A. A. (2004). MrModeltest v2.3. Evolutionary Biology Centre, Uppsala University. Available at http://www.abc.se/~nylander/.

Oberprieler, C., (2005). Temporal and spatial diversification of Circum-Mediterranean Compositae–Anthemidae. *Taxonomy,* 54: 951–966.

Orr, M. R., & Smith, T. B. (1998). Ecology and speciation. *Trends in Ecology and Evolution*, 13: 502–506.

Partridge, T. C., and R. R. Maud. (1987). Geomorphic evolution of southern Africa since the Mesozoic. *South African Journal of Geology,* 90 (2): 179–208.

Partridge, T. C., and R. R. Maud. (2000). Macro-scale geomorphic evolution of southern Africa. In The Cenozoic of southern Africa, edited by T. C. Partridge and R. R. Maud. Oxford, UK: Oxford University Press.

Partridge, T. C. (1997). Cainozoic environmental change in southern Africa, with special emphasis on the last 200 000 years. *Progress in Physical Geography*, *21*(1): 3–22.

Partridge, T. C. (1998). Of diamonds, dinosaurs and diastrophism: 150 years of landscape evolution in southern Africa. *South African Journal of Geology*, *101*(3):167–184.

Partridge, T. C., & Brink, A. B. A. (1967). Gravels and terraces of the lower Vaal River basin. *South African Geographical Journal*, 49:21–38.

Panero, J. L., and V. A. Funk. (2008). The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. *Molecular Phylogenetics and Evolution* 47 (2): 757–82.

Pelser, P.B., Nordenstam, R.B., Kadereit, J.W., Watson, L., (2007). An ITS phylogeny of tribe Senecioneae (Asteraceae) and a new delimitation of Senecio L. *Taxonomy,* 56: 1077–1104.

Phillips, E. P. (1951). The genera of South African flowering plants, Ed. 2. *Botanical Surveys of South Africa Memoirs.* 25

Quezel, P. (1978). Analysis of the flora of the Mediterranean and Saharan Africa. *Annals of the Missouri Botanical Garden*, *65*(2): 479–534.

Rambaut, A. & Drummond, A.J. (2009). Tracer v1.5.0. Available at: http://beast.bio.ed.ac.uk/Tracer.

Raven, P.H. (1973) The evolution of Mediterranean floras. Mediterranean type ecosystems: origin and structure (ed. by F. di Castri and H.A. Mooney), pp. 213–224. Springer, New York

Ree, R. H., Moore, B. R., Webb, C. O., & Donoghue, M. J. (2005). A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution*, *59*(11): 2299–2311.

Ree, R. H., & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, *57*(1): 4–14.

Richardson, J. E., Weitz, F. M., Fay, M. F., Cronk, Q. C., Linder, H. P., Reeves, G., & Chase, M. W. (2001). Rapid and recent origin of species richness in the Cape flora of South Africa. *Nature*, *412*(6843): 181–3.

Roberts, R. P., & Urbatsch, Lowell, E. (2004). Molecular phylogeny of Chrysothamnus and related genera (Asteraceae, Astereae) based on nuclear ribosomal 3' ETS and ITS sequence data. *Systematic Botany*, *29*(1): 199–215.

Rundell, R. J., & Price, T. D. (2009). Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology and Evolution*, 24:394–9.

Sakai, T., Saneyoshi, M., Tanaka, S., Sawada, Y., Nakatsukasa, M., Mbua, E., & Ishida, H. (2010). Climate shift recorded at around 10 Ma in Miocene succession of Samburu Hills, northern Kenya Rift, and its significance**.** *Geological Society of London Special Publication*, 342:109–127.

Sanderson, M. J., & Donoghue, M. J. (1989). Patterns of variation in levels of homoplasy. *Evolution:* 1781–1795.

Sanz, M., Vilatersana, R., Hidalgo, O., Garcia-jacas, N., Susanna, A., Schneeweiss, G. M., &

Vallès, J. (2008). Molecular phylogeny and evolution of floral characters of Artemisia and allies (Anthemideae, Asteraceae): evidence from nrDNA ETS and ITS sequences *Taxon*, 57(February): 66–78.

Sanmartín, I., Anderson, C. L., Alarcon, M., Ronquist, F., & Aldasoro, J. J. (2010). Bayesian island biogeography in a continental setting: the Rand Flora case. *Biology letters*, *6*(5): 703–7.

Schluter, D., Price T., Mooers, A., Ludwig, D. (1997) Likelihood of ancestor states in adaptive radiation. *Evolution* (Lawrence, Kans),51:1699–1711.

Schluter, D. (2000). Ecological character displacement in adaptive radiation. *American Naturalist*, 156:S4-S16.

Schluter, D, (2009). Evidence for ecological speciation and its alternative. *Science*, 323:737–41.

Scott, L., A. Cadman, and I. McMillan. (2006). Early history of Cainozoic Asteraceae along the Southern African west coast. *Review of Palaeobotany and Palynology* 142 (1-2): 47–52.

Scotland, R. W., Olmstead, R. D., & Bennett, J. (2009). Phylogeny reconstruction: the role of morphology. *Systematic Biology*, *52*(4): 539–548.

Schrire, B.D., Lavin, M., Barker, N.P., Cortes-Burns, H., von Senger, I. & Kim, J. (2003). Towards a phylogeny of Indigofera (Leguminosae-Papilionoideae): indentification of major clades and relative ages. *Advances in legume systematics.* Part 10. Higher level systematics (ed. by B.B. Klitgaard and A. Bruneau), pp. 269–302. Royal Botanic Gardens, Kew.

Schrire , B. D., M. Lavin , and G. P. Lewis. (2005). Global distribution patterns of the Leguminosae: insights from recent phylogenies. In I. Friis and H. Balslev [eds.], Plant diversity and complexity patterns: Local, regional and global dimensions, 375–422. Royal Danish Academy of Sciences and Letters, Copenhagen, Denmark.

Sepulchre, P., Ramstein, G., Fluteau, F., Schuster, M., Tiercelin, J.-J., & Brunet, M. (2006). Tectonic uplift and Eastern Africa aridification. *Science (New York, N.Y.)*, *313*(5792): 1419–23.

Shackleton, N. J., & Kennett, J. P. (1975). Paleotemperature history of the Cenozoic and the

initiation of Antarctic glaciation: oxygen and carbon isotope analyses in DSDP Sites 277, 279, and 281. *Initial reports of the deep sea drilling project*, *29:* 743–755.

Shaul, S., Graur, D., (2002). Playing chicken (Gallus gallus): methodological inconsistencies of molecular date estimates due to secondary calibration points. *Gene,* 300: 59–61.

Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, W., Miller, J., … Small, R. L. (2005). The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany*, *92*(1): 142–166.

Siesser, W.G. (1978). Aridification of the Namib Desert, evidence from oceanic cores. In van Zinderen Bakker, E.M., editor, Antarctic glacial history and world palaeoenvironments. Rotterdam: Balkema: 105–13.

Siesser, W.G., (1980). Late Miocene origin of Benguela upwelling system off northern Namibia. *Science,* 208: 283–285.

Simpson, B. B. (1975). Pleistocene changes in the flora of the High Tropical Andes. *Paleobiology*, **1:**273–294.

Smissen, R. D., Galbany-casals, M., & Breitwieser, I. (2011). Ancient allopolyploidy in the everlasting daisies (Asteraceae : Gnaphalieae): Complex relationships among extant clades. *Taxon*, *60:* 1–14.

Starr, J. R., Harris, S. A., & Simpson, D. A. (2003). Potential of the 5’ and 3' ends of the intergenic spacer (IGS) of rDNA in Cyperaceae: new sequences for lower-level phylogenies in sedges with an example from Uncinia Pers. *International Journal of Plant Sciences*, *164*(2): 213–227.

Suc, J.P. (1984) Origin and evolution of the Mediterranean vegetation and climate in Europe. *Nature*, 307: 429–432

Swofford, D. L. 2002. PAUP* version 4.0. Phylogenetic Analysis Using Parsimony (and Other Methods).

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three

non-coding regions of chloroplast DNA. *Plant Molecular Biology*, *17*(5): 1105–1109.

Taylor, H.C. (1978) Capensis. Biogeography and ecology of southern Africa (ed. by M.J.A. Werger), pp. 171–229. Junk, The Hague.

Thiv, M., van der Niet, T., Rutschmann, F., Thulin, M., Brune, T., & Linder, H. P. (2011). Old-New World and trans-African disjunctions of Thamnosma (Rutaceae): intercontinental long-distance dispersal and local differentiation in the succulent biome. *American Journal of Botany*, *98*(1): 76–87.

Thorne, R. E. (1972). Major disjunctions in the geographic ranges of seed plants. *Quarterly Review of Biology, 47*: 365–411.

Thompson, J.D. (2005) Plant evolution in the Mediterranean. Oxford University Press, Oxford.

Trauth, M. H., Larrasoaña, J. C., & Mudelsee, M. (2009). Trends, rhythms and events in Plio-Pleistocene African climate. *Quaternary Science Reviews*, *28*(5-6): 399–411.

Tyson, P. D., and T. C. Partridge. (2000). Evolution of Cenozoic climates. In *The Cenozoic of southern Africa*, edited by T. C. Partridge and R. R. Maud. Oxford: Oxford University Press.

Uys, E., & Cron, G. V. (2013). Relationships and evolution in the Drakensberg near-endemic genus ,Craterocapsa (Campanulaceae). *South African Journal of Botany*, *86:* 79–91.

Van Dam, J.A. (2006) Geographic and temporal patterns in the late Neogene (12–3 Ma) aridification of Europe: the use of small mammals as paleoprecipitation proxies. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 238: 190–218.

Van Rooyen, M.W. (1999). Functional aspects of short-lived plants. Chapter 7. In W.R.J. Dean and S.J. Milton (eds.) The Karoo: Ecological patterns and processes. Cambridge University Press.

Van Zinderen Bakker, E. M. (1975). The origin and paleoenvironment of the Namib desert biome. *Journal of Biogeography*, *2*(5): 65–73.

Van Zinderen Bakker, E. M. (1981). The high mountains of Lesotho – a botanical paradise. *Veld & Flora,* 67: 106–108.

Verboom, G. A., Linder, H. P., & Stock, W. D. (2003). Phylogenetics of the grass genus Ehrharta: evidence for radiation in the summer-arid zone of the South African Cape. *Evolution*, *57*(5): 1008–1021.

Verboom, G. A., Archibald, J. K., Bakker, F. T., Bellstedt, D. U., Conrad, F., Dreyer, L. L., … Nowell, T. L. (2009). Origin and diversification of the Greater Cape flora : Ancient species repository, hot-bed of recent radiation, or both? *Molecular Phylogenetics and Evolution*, *51:* 44–53.

Verdcourt, B. (1969). The arid corridor between the north-east and south- west areas of Africa, *Palaeoecology of Africa,* 4: 140–144.

Ward, J., Bayer, R.J., Breitwieser, I., Smissen, R., Galbany-Casals, M. & Unwin, M. Ch. 36: Gnaphalieae. (2009). In *Systematics, Evolution and Biogeography of Compositae*. Edited by Funk V.A., Susanna A., Stuessy T.F. & Bayer R.J. International Association for Plant Taxonomy, Vienna, 539–588.

Watson, A., & Williams, D. P. (1985). Early Pleistocene river gravels in Swaziland and their geomorphological and structural significance. *Zeitschrift fur Geomorphologie, New Series*, 29:71–87.

Weimark, H. (1941). Phytogeographic groups, centres and intervals within the Cape flora, *Lunds Universitets Arrskrift, N.F.,* 2: 37: 261–281.

White, F. (1978). The Afromontane Region. In: Werger, M.J.A. & Van Bruggen, A.C. (eds), *Biogeography and ecology of southern Africa,* pp. 463–513. Dr W. Junk, The Hague.

White, F. (1983). *Vegetation of Africa: a descriptive memoir to accompany the UNESCO/AETFAT/UNSO vegetation map of Africa*, UNESCO, Paris.

White, T.J., Bruns, T., Lee, S., Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications*. Edited by Innis M.A., Gelfand D.H., Sninsky J.J., White T.J. New York: Academic Press Inc., 315–322.

Wiens, J. J. (2004). Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. *Evolution,58*(1): 193–197.

Wiens, J. J., Ackerly, D. D., Allen, A. P., Anacker, B. L., Buckley, L. B., Cornell, H. V, … Stephens, P. R. (2010). Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology letters*, *13*(10): 1310–24.

Wiens, J. J. (2001). Character analysis in morphological phylogenetics : problems and solutions. *Systematic Biology*, *50*(5): 689–699.

Wiens, J. J. (2006). Missing data and the design of phylogenetic analyses. *Journal of Biomedical Informatics*, *39:* 34–42.

Wild, H. (1968). Phytogeography in south central Africa. *Kirkia* 6: 197–222.

Wolfe, K.H., Li, W.-H. & Sharp, P.M. (1987). Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast and nuclear DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 84: 9054–9058

Wolfenden, E., Ebinger, C., Yirgu, G., Deino, A., & Ayalew, D. (2004). Evolution of the northern Main Ethiopian rift: birth of a triple junction. *Earth and Planetary Science Letters*, *224*(1-2): 213–228.

Yang, Z., & Rannala, B. (1997). Monte Carlo Method A Markov Chain I-I. *Molecular Biology and Evolution*, *14*(7): 717–724.

Yang, F.-S., Qin, A.-L., Li, Y.-F., & Wang, X.-Q. (2012). Great genetic differentiation among populations of Meconopsis integrifolia and its implication for plant speciation in the Qinghai-Tibetan Plateau**.** *PloS one*, 7:e37196.

Yates, M.J., Verboom, G.A., Rebelo, A.G. & Cramer, M.D. (2010). Ecophysiological significance of leaf size variation in Proteaceae from the Cape Floristic Region**.** *Functional Ecology,* 24**:** 485–492.

Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K. (2001). Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292: 686–693

Zachos, J. C., G. R. Dickens, and R. E. Zeebe. (2008). An Early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature* 451: 279–283.

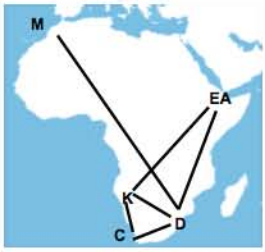
Zwickl, D.J., Hillis, D.M., (2002). Increased taxon sampling greatly reduces phylogenetic error. *Systematic Biology,* 51, 588–594.

Xu, T., Abbott, R. J., Milne, R. I., Mao, K., Du, F. K., Wu, G., Ciren, Z., et al. (2010). Phylogeography and allopatric divergence of cypress species (Cupressus L.) in the Qinghai-Tibetan Plateau and adjacent regions. *BMC Evolutionary Biology*, 10: 194.

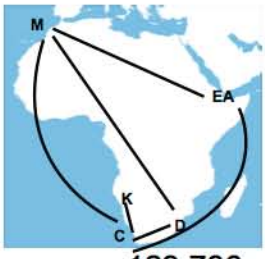
## Appendix 1. Alternative dispersal scenarios which were tested in Lagrange but not shown in Chapter Three.

The associated –*ln* likelihood score is indicated below the map. M – circum-Mediterranean; C – GCFR; D – Drakensberg; K – Nama Karoo & Namibia; EA – East African Highlands.

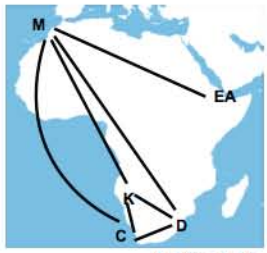
Two further models not shown (due to their extremely poor performance) are those restricting dispersal between C and D and between C and K.



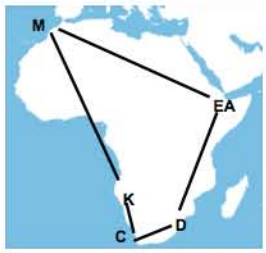
-118.006



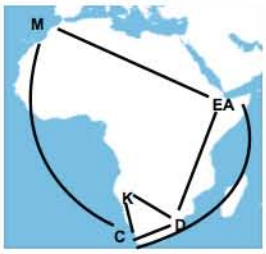
-129.786



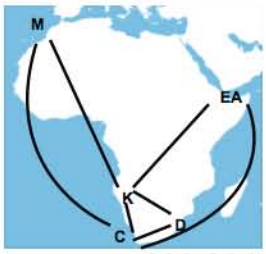
-139.639



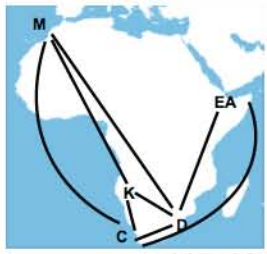
-114.172



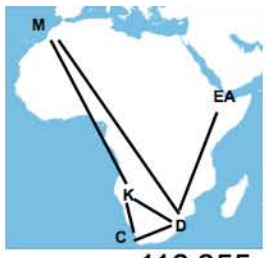
-116.253



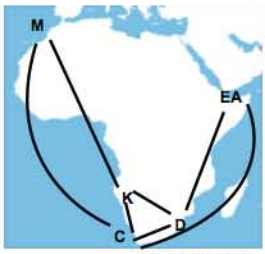
-130.884



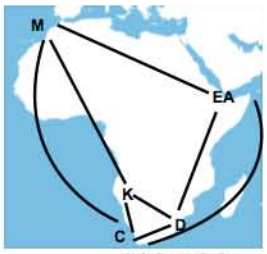
-117.13



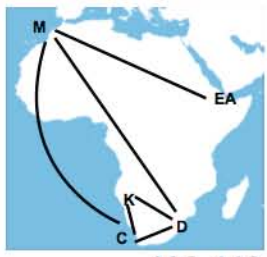
-116.355



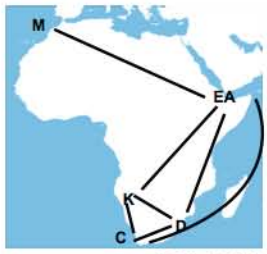
-115.596



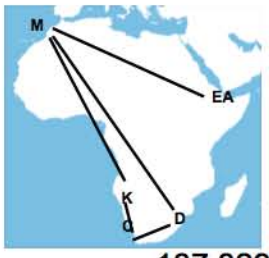
-116.538



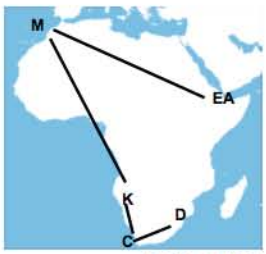
-138.443



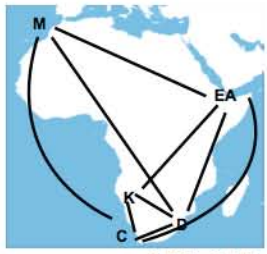
-122.216



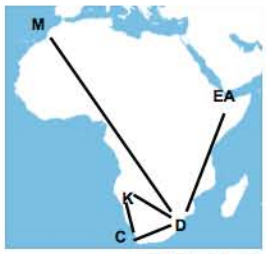
-137.829



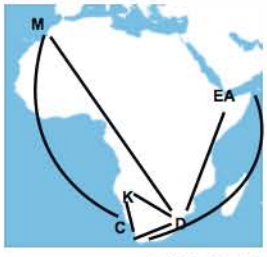
-160.288



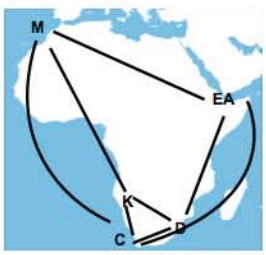
-117.217



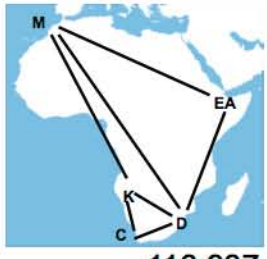
-117.355



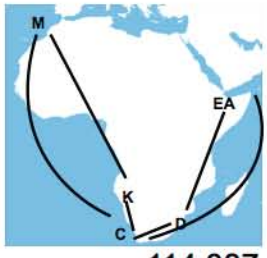
-115.935



-115.596



-116.937



-114.087